Ultra
617099

```
=> e rim2/ct 5
E#   FREQUENCY     AT      TERM
--   ---------     --      ----
E1           0      2      RIM/CT
E2           0      2      RIM (MOLDING)/CT
E3           0        --> RIM2/CT
E4           1      6      RIMACEPHALUS/CT
E5           1      6      RIMACEPHALUS ARECEPTA/CT

=> e rim 2/ct 5
E#   FREQUENCY     AT      TERM
--   ---------     --      ----
E1           0      2      RIM/CT
E2           0      2      RIM (MOLDING)/CT
E3           0        --> RIM 2/CT
E4           1      6      RIMACEPHALUS/CT
E5           1      6      RIMACEPHALUS ARECEPTA/CT

=> e e1+all/ct
E1           0    -->  RIM/CT
E2                  USE  Molding of plastics and rubbers (L) reaction
                         injection/CT
*********  END***

=> fil reg;e rim/cn 5
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.65       0.80

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http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf


E1           1      RILUTEK/CN
E2           1      RILUZOLE/CN
E3           0 --> RIM/CN
E4           1      RIM 1/CN
E5           1      RIM 83/CN

=> fil medl,biosis,caplus,embase,jicst,wpids
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.31       1.11

FILE 'MEDLINE' ENTERED AT 12:47:13 ON 30 NOV 2001


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=> s rim or rim2
L1 5637 FILE MEDLINE
L2 5176 FILE BIOSIS
L3 10857 FILE CAPLUS
L4 5438 FILE EMBASE
L5 2000 FILE JICST-EPLUS
L6 61686 FILE WPIDS

TOTAL FOR ALL FILES
L7 90794 RIM OR RIM2

=> s l7(l)(g protein or rab3 or gdp or gtp or gdp gtp or gefii or camp dependent or protein kinase a or pka or exocytosis(5a)(neuron! or endocrine))
L8 28 FILE MEDLINE
L9 40 FILE BIOSIS
L10 44 FILE CAPLUS
L11 25 FILE EMBASE
L12 1 FILE JICST-EPLUS
L13 5 FILE WPIDS

TOTAL FOR ALL FILES
L14 143 L7(L)(G PROTEIN OR RAB3 OR GDP OR GTP OR GDP GTP OR GEFII OR CAMP DEPENDENT OR PROTEIN KINASE A OR PKA OR EXOCYTOSIS(5A)(NEUR ON! OR ENDOCRINE))

=> dup rem l14
PROCESSING COMPLETED FOR L14
L15 62 DUP REM L14 (81 DUPLICATES REMOVED)

=> d 1-62 cbib abs

L15 ANSWER 1 OF 62 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
2001:261133 Document No. 134:291132 Protein and cDNA sequences of mouse protein Rim2 and diagnostic and therapeutic uses thereof. Seino, Susumu; Shibasaki, Tadao; Ozaki, Nobuaki (JCR Pharmaceuticals Co., Ltd., Japan). Eur. Pat. Appl. EP 1090986 A1 20010411, 42 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2000-116148 20000731. PRIORITY: JP 1999-288372 19991008.
AB The present invention provides protein and cDNA sequences of mouse protein **Rim2**, which is a novel isoform of **Rim**, i.e., a protein that interacts with a low mol. **G protein Rab3** and is proposed to serve as a regulator of **Rab3**-dependent synaptic vesicle fusion, and which specifically interacts with the

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GDP/GTP exchange factor (**GEFII**; a cAMP sensor). The full-length novel mouse protein **Rim2** sequenced by the present inventors, which consists of 1590 amino acid residues, was found to have 61.6 % identity with rat Rim1. A zinc finger, PDZ and two C2 domains were found highly conserved between Rim1 and **Rim2**. The invention also relates a mutant protein has an amino acid sequence with one or more amino acids deleted, substituted, inserted or added relative to the amino acid sequence of wild type mouse protein **Rim2** and which has a property to interact with **GDP/GTP** exchange factor II. The present invention further relates to elucidation of the mechanisms of intracellular vesicle transport and secretion, and to the novel protein **Rim2** which is useful in diagnosis of endocrine-related diseases or neuropathy and in development of agents for prevention and treatment thereof, the gene encoding **Rim2** and an antibody addressed to **Rim2** protein.

L15 ANSWER 2 OF 62 MEDLINE DUPLICATE 2

2001479158 Document Number: 21413925. PubMed ID: 11438518. Direct interaction of the **Rab3** effector **RIM** with Ca2+ channels, SNAP-25, and synaptotagmin. Coppola T; Magnin-Luthi S; Perret-Menoud V; Gattesco S; Schiavo G; Regazzi R. (Institut de Biologie Cellulaire et de Morphologie, University of Lausanne, 1005 Lausanne, Switzerland.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Aug 31) 276 (35) 32756-62. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB To define the role of the **Rab3**-interacting molecule **RIM** in exocytosis we searched for additional binding partners of the protein. We found that the two C(2) domains of **RIM** display properties analogous to those of the C(2)B domain of synaptotagmin-I. Thus, **RIM**-C(2)A and **RIM**-C(2)B bind in a Ca(2+)-independent manner to alpha1B, the pore-forming subunit of N-type Ca(2+) channels (EC(50) = approximately 20 nm). They also weakly interact with the alpha1C but not the alpha1D subunit of L-type Ca(2+) channels. In addition, the C(2) domains of **RIM** associate with SNAP-25 and synaptotagmin-I. The binding affinities for these two proteins are 203 and 24 nm, respectively, for **RIM**-C(2)A and 224 and 16 nm for **RIM** -C(2)B. The interactions of the C(2) domains of **RIM** with SNAP-25 and synaptotagmin-I are modulated by Ca(2+). Thus, in the presence of Ca(2+) (EC(50) = approximately 75 microm) the interaction with synaptotagmin-I is increased, whereas SNAP-25 binding is reduced. Synaptotagmin-I binding is abolished by mutations in two positively charged amino acids in the C(2) domains of **RIM** and by the addition of inositol polyphosphates. We propose that the **Rab3** effector **RIM** is a scaffold protein that participates through its multiple binding partners in the docking and fusion of secretory vesicles at the release sites.

L15 ANSWER 3 OF 62 MEDLINE DUPLICATE 3

2001169940 Document Number: 21167768. PubMed ID: 11134008. A direct inhibitory role for the Rab3-specific effector, Noc2, in Ca2+-regulated exocytosis in neuroendocrine cells. Haynes L P; Evans G J; Morgan A; Burgoyne R D. (Physiological Laboratory, University of Liverpool, Crown Street, Liverpool L69 3BX, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Mar 30) 276 (13) 9726-32. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Rab proteins comprise a family of GTPases, conserved from yeast to mammals, which are integral components of membrane trafficking pathways. Rab3A is a neural/neuroendocrine-specific member of the Rab family involved in Ca(2+) -regulated exocytosis, where it functions in an inhibitory capacity controlling recruitment of secretory vesicles into a releasable pool at the plasma membrane. The effector by which Rab3A exerts its inhibitory effect is unclear as the Rab3A effectors Rabphilin and

RIM have been excluded from for this role. One putative Rab3A effector in dense-core granule exocytosis is the cytosolic zinc finger protein, Noc2. We have established that overexpression of Noc2 in PC12 cells has a direct inhibitory effect upon Ca(2+)-triggered exocytosis in permeabilized cells. We demonstrate specific nucleotide-dependent binding of Noc2 to Rab3A and show that the inhibition of exocytosis is dependent upon this interaction since Rab3A binding-deficient mutants of Noc2 do not inhibit exocytosis. We propose that Noc2 may be a negative effector for Rab3A in regulated **exocytosis** of dense-core granules from **endocrine** cells.

L15 ANSWER 4 OF 62 CAPLUS COPYRIGHT 2001 ACS

2001:616710 Multimodal inclusion complexes between barbiturates and 2-hydroxypropyl-.beta.-cyclodextrin in aqueous solution: isothermal titration microcalorimetry, 13C NMR spectrometry, and molecular dynamics simulation. Aki, Hatsumi; Niiya, Tokihiro; Iwase, Yukiko; Yamamoto, Magobei (Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, 814-0180, Japan). J. Pharm. Sci., 90(8), 1186-1197 (English) 2001. CODEN: JPMSAE. ISSN: 0022-3549. Publisher: Wiley-Liss, Inc..

AB Multiple types (structures) of inclusion complexes between barbiturates and 2-hydroxypropyl-.beta.-cyclodextrin (HPCD) were evaluated by isothermal titrn. microcalorimetry and 13C NMR spectroscopy. The geometries of the inclusion complexes were suggested by mol. dynamics simulation. Barbituric acid (BA), barbital (B), amobarbital (AB), pentobarbital (PB), secobarbital (SB), cyclobarbital (CB), and phenobarbital (PHB) were used as barbiturates with different substituents on the barbituric acid ring and compared for inclusion types in aq. soln. The assocn. consts. (K), stoichiometries, and thermodn. parameters change in free energy (.DELTA.G) change in enthalpy (.DELTA.H), and change in entropy [.DELTA.S] for each type of complex were detd. from the calorimetric data. The inclusion complexation was largely entropy driven because of hydrophobic interactions. The values of K increased in the order BA<B<AB<PB<SB<CB<PHB. Barbiturates, except B and BA, form two types of inclusion complex with a 1:1 stoichiometry in the un-ionized forms. The first type of inclusion complex with high affinity (K1) was characterized by small neg. values of .DELTA.H1 and large pos. .DELTA.S1, where the substituent R2 of the barbiturate was initially inserted into the cavity of HPCD through hydrophobic interactions. There was a good relationship between .DELTA.G1 obtained from the calorimetric data for the first type of inclusion complex and .DELTA.GR2 calcd. from the changes in 13C NMR (NMR) chem. shifts for the substituent R2 of barbiturates. These types were very stable in aq. soln. at various pHs. The second type of complex, with low affinity (K2), was characterized by large neg. values of .DELTA.H2 and small pos. .DELTA.S2, reflecting van der Waals' interactions in the un-ionized forms of barbiturates at pH values less than **pKa** . The values of K2 were markedly decreased to <103 M-1 as the barbiturates were ionized over pH 8. Thus, in the second type, the barbituric acid ring contributed to forming the complexes. The geometries were stabilized by hydrogen bond formation between the hetero atoms in the barbituric acid ring and the secondary hydroxyl groups on the **rim** of the cyclodextrin. The 13C NMR chem. shifts of C4 and C6 carbons in the barbituric acid ring were moved upfield significantly by the inclusion complexation. On the other hand, B and BA could form only one type of complex, the lid-type supramol. complex with small assocn. consts.

L15 ANSWER 5 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS

2001:520198 Document No.: PREV200100520198. RIMs as signalling scaffolds at the active zone. Schoch, S. (1); Castillo, P. E.; Mukhergee, K. (1); Wang, Y. (1); Malenka, R. C.; Sudhof, T. C. (1). (1) Center for Basic Neuroscience, Howard Hughes Medical Institute, UT Southwestern Medical Center, Dallas, TX USA. Society for Neuroscience Abstracts, (2001) Vol.

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27, No. 1, pp. 1016. print. Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001 ISSN: 0190-5295. Language: English. Summary Language: English.

AB The active zone of a synapse is a specialized region of the presynaptic plasma membrane localized exactly opposite of the postsynaptic reception machinery. It is involved in mediating calcium-triggering of neurotransmitter release and in processes underlying various forms of short- and long-term synaptic plasticity. In recent years a number of active zone proteins have been identified but very little is known about their function and their interactions with synaptic vesicles. **RIMs** are active zone proteins that were initially identified as putative effectors for the synaptic vesicle **GTP**-binding protein rab3A. The structure of **RIM** is composed of several known protein-protein interaction domains, including PDZ-domain, zinc-finger domain and two C2-domains. We analyzed these domains for their ability to interact with proteins that are localized to the active zone or synaptic vesicles. To study the function of RIM1-alpha we generated a mouse model in which RIM1-alpha expression has been deleted. Biochemical and histochemical analysis points to a role for **RIM**'s as presynaptic scaffolding proteins and integrators of various signals at the active zone.

L15 ANSWER 6 OF 62 MEDLINE DUPLICATE 4

2001528561 Document Number: 21458927. PubMed ID: 11559854. A post-docking role for active zone protein Rim. Koushika S P; Richmond J E; Hadwiger G; Weimer R M; Jorgensen E M; Nonet M L. (Department of Anatomy and Neurobiology, Washington University School of Medicine, 660 S. Euclid Avenue, Saint Louis, Missouri 63110, USA.) NATURE NEUROSCIENCE, (2001 Oct) 4 (10) 997-1005. Journal code: DA8; 9809671. ISSN: 1097-6256. Pub. country: United States. Language: English.

AB Rim1 was previously identified as a **Rab3** effector localized to the presynaptic active zone in vertebrates. Here we demonstrate that C. elegans unc-10 mutants lacking **Rim** are viable, but exhibit behavioral and physiological defects that are more severe than those of **Rab3** mutants. **Rim** is localized to synaptic sites in C. elegans, but the ultrastructure of the presynaptic densities is normal in **Rim** mutants. Moreover, normal levels of docked synaptic vesicles were observed in mutants, suggesting that **Rim** is not involved in the docking process. The level of fusion competent vesicles at release sites was reduced fivefold in **Rim** mutants, but calcium sensitivity of release events was unchanged. Furthermore, expression of a constitutively open form of syntaxin suppressed the physiological defects of **Rim** mutants, suggesting **Rim** normally acts to regulate conformational changes in syntaxin. These data suggest **Rim** acts after vesicle docking likely via regulating priming.

L15 ANSWER 7 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS

2001:497843 Document No.: PREV200100497843. High expression of human RIM gene in neuroblastomas with favorable biologies. Aoyama, M. (1); Asai, K.; Shishikura, T. (1); Ohira, M. (1); Inuzuka, H. (1); Morohashi, A. (1); Kato, T.; Nakagawara, A. (1). (1) Division of Biochemistry, Chiba Cancer Center Research Institute, Chiba Japan. Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 715. print. Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001 ISSN: 0190-5295. Language: English. Summary Language: English.

AB Differential screening between human neuroblastomas with favorable biologies, well-differentiated tumors, and those with unfavorable biologies, poorly differentiated, using full-length-enriched neuroblastoma cDNA libraries identified a novel gene, nbla-761, that was highly homologous to the rat **Rab3**-interacting molecules (**RIM**). To characterize the gene, two forms of the full-length cDNAs were

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isolated. Their predicted products had multiple domains, including a PDZ domain and C2 domains near the COOH terminus and one pair of Cys4 zinc finger near the NH2 terminus. All of these domains were common to rat **RIM**, therefore nbla-761 was suggested to be a human homologue of **RIM** gene. Reverse transcription-PCR (RT-PCR) analysis of various human tissues showed that **RIM** was expressed in the brain, lung, trachea, and testis. To confirm the differential expression in neuroblastomas, the expression of **RIM** was investigated in 105 human primary tumors by a RT-PCR and a real-time detection PCR (RTD-PCR). The expression level of **RIM** mRNA correlated with favorable tumor stage (I, II, and IVs vs. III and IV; p=0.0025), younger age (<1 year vs. >1 year; p=0.0094), normal N-myc copy number (p=0.0218), high TRKA expression (p=0.0014), and DNA ploidy (aneuploidy vs. diploidy; p=0.0169). The most significant correlation was with the high TRKA expression. We propose that human **RIM** is a novel favorable prognostic factor like TRKA in human neuroblastomas. **RIM** may be relative to the differentiation of neuroblastomas.

L15 ANSWER 8 OF 62 MEDLINE DUPLICATE 5
2001563529 Document Number: 21521551. PubMed ID: 11640918.
Characterization of rabphilin phosphorylation using phospho-specific antibodies. Lonart G; Sudhof T C. (Center for Basic Neuroscience, Department of Molecular Genetics and Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, Room NA4.118, 75390-9111, Dallas, TX, USA.) NEUROPHARMACOLOGY, (2001 Nov) 41 (6) 643-9. Journal code: NZB; 0236217. ISSN: 0028-3908. Pub. country: England: United Kingdom. Language: English.

AB Rab3A is a **GTP**-binding protein of synaptic vesicles that regulates neurotransmitter release and cycles on and off synaptic vesicles as a function of exocytosis. Rab3A presumably functions via **GTP** -dependent interactions with effectors. Two putative rab3A effectors have been described in neurons, rabphilin which is a soluble protein that moves onto and off synaptic vesicles in concert with rab3A, and **RIM** which is an active zone protein that only binds to rab3A on docked vesicles. Rabphilin is an abundant, evolutionarily conserved protein whose function has remained enigmatic since a knockout of rabphilin does not display the functional deficiencies observed in the rab3A knockout. However, previous studies have shown that rabphilin is phosphorylated by **protein kinase A** and CaM Kinase II, suggesting that it may have a regulatory role. In the present study, we have examined the site and regulation of rabphilin phosphorylation in living nerve terminals using phospho-specific antibodies raised against phospho-serine(234) of rabphilin. With these antibodies, we demonstrate that rabphilin is physiologically phosphorylated on serine(234), and that soluble rabphilin which is not bound to rab3A on synaptic vesicles is the primary target. However, different from synapsins which are induced to dissociate from synaptic vesicles by **PKA** phosphorylation, phosphorylation of rabphilin is not instrumental for dissociating rabphilin from synaptic vesicles. Our data support the notion that dissociated rabphilin is a synaptic phosphoprotein in vivo that may play a role in the regulation of nerve terminal protein-protein interactions.

L15 ANSWER 9 OF 62 CAPLUS COPYRIGHT 2001 ACS
2001:228088 Document No. 135:107314 Crown ethers derived from bicyclocalix[4]arenes as chromoionophores. Wasikiewicz, Wojciech; Slaski, Michal; Rokicki, Gabriel; Bohmer, Volker; Schmidt, Christian; Paulus, Erich F. (Faculty of Chemistry, Warsaw University of Technology, Warsaw, PL-00664, Pol.). New J. Chem., 25(4), 581-587 (English) 2001. CODEN: NJCHE5. ISSN: 1144-0546. Publisher: Royal Society of Chemistry.

AB The synthesis of calix[4]arenes in which opposite phenolic units are connected by a poly(oxyethylene) bridge at the narrow **rim** and a 2,6-dimethylene-4-nitrophenol bridge at the wide **rim** is

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reported. For two derivs. with tetra- (I) and penta(oxyethylene) (II) bridges UV-Vis spectrophotometric studies were carried out in buffered soln. in the presence of alkali metal ions. Their complexation was assocd. with changes in their UV-Vis spectra, esp. with an increase of the absorption band at 450 nm. For I and II this was most intense in the presence of potassium and cesium ions, resp., indicating that the calixarene with the shorter crown ether bridge is selective towards potassium ions and that with the longer bridge towards cesium ions. The X-ray structure anal. of II shows a pinched cone conformation for the 1,3-calixcrown part. The p-nitrophenol unit is parallel to one of the unbridged tert-Bu phenol units. Its hydroxyl group points into the cavity, in agreement with comparatively high **pKa** values. Mol. mechanics was used to model the geometry of the potassium and cesium complexes.

L15 ANSWER 10 OF 62 MEDLINE DUPLICATE 6
2001530795 Document Number: 21461363. PubMed ID: 11576649. Dual use of the transcriptional repressor (CtBP2)/ribbon synapse (RIBEYE) gene: how prevalent are multifunctional genes?. Piatigorsky J. (Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, 20892, Bethesda, MD, USA.) TRENDS IN NEUROSCIENCES, (2001 Oct) 24 (10) 555-7. Journal code: WEL; 7808616. ISSN: 0166-2236. Pub. country: England: United Kingdom. Language: English.
AB Vertebrates have ribbon synapses in the retina and in other sensory structures that are specialized for rapid, tonic release of synaptic vesicles (1). The lamellar sheets of the ribbon situated at right angles to the plasma membrane are lined with synaptic vesicles that undergo exocytosis under the influence of Ca(2+). Synaptic ribbons act as a conveyer belt to accelerate the release of this ready supply of synaptic vesicles at the presynaptic membranes. Although the protein composition of the terminals of ribbon synapses is generally similar to that of ordinary synapses in nervous tissue, much less is known about the composition of the ribbons themselves. **RIM**, a universal component of presynaptic active zones that interacts with **rab3** on the synaptic vesicle, has been localized to the ribbons (2). In addition, the kinesin motor protein, KIF3A, is associated with the ribbons and other organelles in presynaptic nerve terminals (3). Recently, a approximately 120 kDa protein called RIBEYE has been identified in purified ribbons of bovine retina. The RIBEYE cDNA was cloned and its gene identified in the database.

L15 ANSWER 11 OF 62 CAPLUS COPYRIGHT 2001 ACS
2001:241880 Document No. 134:217282 Regulation of insulin secretion by a novel cAMP sensor. Miki, Takashi; Seino, Susumu (Dep. Mol. Med., Chiba Univ. Grad. Sch. Med., Japan). Saishin Igaku, 56(3), 488-492 (Japanese) 2001. CODEN: SAIGAK. ISSN: 0370-8241. Publisher: Saishin Igakusha.
AB A review with 11 refs., on identification of cAMP-**GEFII** as a cAMP sensor, identification of **Rim2** interacted with cAMP-**GEFII**, and roles of cAMP-**GEFII** in cAMP-induced exocytosis and **cAMP-dependent** insulin secretion.

L15 ANSWER 12 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
2001:481652 Document No.: PREV200100481652. The death domain of Rab3 guanine nucleotide exchange protein is required for GDP/GTP exchange activity in living cells. Regazzi, R. (1); Perret-Menoud, V. (1); Gattesco, S. (1); Magnin, S. (1); Pombo, I.; Blank, U.; Epping-Jordan, M. P.; Coppola, T. (1). (1) IBCM, University of Lausanne, Lausanne Switzerland. Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1, pp. 330. print. Meeting Info.: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001 ISSN: 0190-5295. Language: English. Summary Language: English.
AB **Rab3** GTPases regulate **exocytosis** of **neurons**,

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endocrine and exocrine cells. In this study, we developed a system to measure the guanine nucleotide status of **Rab3** proteins in living cells. The assay is based on the ability of **RIM** (**Rab3** Interacting Molecule) to pull-down selectively the **GTP**-bound form of **Rab3**. Using this system, we found that apprx20% of wild type Rab3A, -B, -C or -D transfected in the insulin-secreting cell line HIT-T15 is in the **GTP**-bound conformation. The pool of activated **Rab3** is decreased under conditions that stimulate exocytosis or by co-expression of **Rab3** -GAP (GTPase Activating Protein). In contrast, co-expression of Mss4 or **Rab3**-GEP (Guanine nucleotide Exchange Protein) increases apprx3-fold the **GTP**-bound pool of **Rab3** isoforms. **Rab3**-GEP is highly homologous to MADD, a death domain-containing protein that associates with the type 1 tumor necrosis factor receptor. We observed that the death domain of **Rab3**-GEP is involved in intramolecular interactions and that deletions or mutations that affect this domain of the protein impairs the nucleotide exchange activity toward **Rab3**. We propose that the death domain of **Rab3**-GEP acts as a molecular switch that, by exchanging its binding partners, coordinates multiple functions of the protein.

L15 ANSWER 13 OF 62 CAPLUS COPYRIGHT 2001 ACS
2001:293843 Document No. 134:293140 Modulation of neurotransmitter release and synaptic plasticity. Sasaki, Takuya; Orita, Satoshi; Miyoshi, Jun; Takai, Yoshimi (Dep. Biochem., The Univ. Tokushima Sch. Med., 3-18-15 Kuramoto-cho, Tokushima, 770-8503, Japan). No no Kagaku, 23(4), 297-306 (Japanese) 2001. CODEN: NNOKFZ. ISSN: 1343-4144. Publisher: Seiwa Shoten.
AB A review with 39 refs., on (1) roles of SNARE system in neurotransmitter release, (2) regulation of Rab3A small **G protein** and its function in neurotransmitter release, (3) involvement of Rab3A and related mols. in memory and learning, (4) functions of Rab GDI and Rab GEP in synaptic transmission, (5) discovery of novel proteins having two C2 domains (Rabphilin-3, **Rim**, Doc2.alpha., etc.) and their functions in transmitter release, (6) roles of Doc2.alpha. and Munc13 in the regulation of synaptic vesicular transport, and (7) interaction among Doc2.alpha. system, Rab3A system, and SNARE system in the regulation of transmitter release and higher neuronal functions.

L15 ANSWER 14 OF 62 CAPLUS COPYRIGHT 2001 ACS
2001:810566 Chiral separation of 9-fluorenylmethyl chloroformate- and dansyl chloride-derivatized d,l-serine by .gamma.-cyclodextrin-bonded high-performance liquid chromatography. Kim, Tae-Young; Kim, Hie-Joon (School of Chemistry & Molecular Engineering, Seoul National University, 151-747, Seoul, S. Korea). J. Chromatogr., A, 933(1-2), 99-106 (English) 2001. CODEN: JCRAEY. ISSN: 0021-9673. Publisher: Elsevier Science B.V..
AB When acetate buffer was used in chiral sepn. of d,l-serine derivs. using a .gamma.-cyclodextrin (CD) column, both retention factor and resoln. were high below the **pKa** of acetic acid and decreased sharply as the pH approached the **pKa**. A similar result was obtained by increasing the buffer concn. at a fixed pH. These observations suggest that hydrogen bonding interaction between the carboxylate group of the amino acid and the secondary hydroxyl groups at the CD **rim** plays an important role in chiral sepn. and is disrupted by the buffer anion.

L15 ANSWER 15 OF 62 CAPLUS COPYRIGHT 2001 ACS
2001:364368 Document No. 135:162549 cAMP sensor and insulin secretion. Ozaki, Nobuaki; Shibazaki, Tadao; Kiyono, Susume (Graduate School of Medicine, Chiba University, Japan). Bunshi Tonyobyogaku no Shinpo 15-19 (Japanese) 2001. CODEN: BTSHFO. Publisher: Kanehara Shuppan.
AB A review with 10 refs., on the title topic, discussing target mols. for cAMP in secretory cell exocytosis, with emphasis on role of cAMP target

mols. (e.g. cAMP-**GEFII** and **Rim2**) in the **protein kinase A**-dependent and -independent pancreatic islet insulin secretion.

L15 ANSWER 16 OF 62 MEDLINE DUPLICATE 7
2000387151 Document Number: 20347919. PubMed ID: 10748113. The **RIM**/NIM family of neuronal C2 domain proteins. Interactions with **Rab3** and a new class of Src homology 3 domain proteins. Wang Y; Sugita S; Sudhof T C. (Center for Basic Neuroscience, Department of Molecular Genetics and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9111, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Jun 30) 275 (26) 20033-44. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB RIM1 is a putative effector protein for Rab3s, synaptic **GTP**-binding proteins. RIM1 is localized close to the active zone at the synapse, where it interacts in a **GTP**-dependent manner with **Rab3** located on synaptic vesicles. We now describe a second **RIM** protein, called **RIM2**, that is highly homologous to RIM1 and also expressed primarily in brain. Like RIM1, **RIM2** contains an N-terminal zinc finger domain that binds to **Rab3** as a function of **GTP**, a central PDZ domain, and two C-terminal C(2) domains that are separated by long alternatively spliced sequences. Unexpectedly, the 3'-end of the **RIM2** gene produces an independent mRNA that encodes a smaller protein referred as NIM2. NIM2 is composed of a unique N-terminal sequence followed by the C-terminal part of **RIM2**. Data bank searches identified a third **RIM**/NIM-related gene, which encodes a NIM isoform referred to as NIM3; no **RIM** transcript from this gene was detected. To test if NIMs, like **RIMs**, may function in secretion, we investigated the effect of NIM3 on calcium-triggered exocytosis in PC12 cells. NIM3 induced a dramatic increase in calcium-evoked exocytosis (50%), with no significant effect on base-line release, suggesting that NIMs, like **RIMs**, regulate exocytosis The combination of conserved and variable sequences in **RIMs** and NIMs indicates that the individual domains of these proteins provide binding sites for interacting molecules during exocytosis, as shown for the zinc finger domain of **RIM**, which binds to **GTP**-bound Rab3s. To search for additional interacting proteins for **RIMs**, we employed yeast two-hybrid screens with the C-terminal half of RIM1. Two members of a new family of homologous brain proteins, referred to as **RIM**-binding proteins (**RIM**-BPs), were identified. **RIM**-BPs bind to **RIM** in yeast two-hybrid and GST pull-down assays, suggesting a specific interaction. In **RIMs**, the binding site for **RIM**-BPs consists of a conserved proline-rich sequence between the two C(2) domains, N-terminal to the beginning of NIMs. **RIM**-BPs are composed of multiple domains, including three fibronectin type III-domains and three Src homology 3 domains, of which the second Src homology 3 domain binds to **RIMs**. With the **RIM**-BPs, we have identified a partner for **RIMs** that may bind to **RIMs** at the synapse in addition to **Rab3**.

L15 ANSWER 17 OF 62 MEDLINE DUPLICATE 8
2001132056 Document Number: 20512528. PubMed ID: 11056535. cAMP-GEFII is a direct target of cAMP in regulated exocytosis. Ozaki N; Shibasaki T; Kashima Y; Miki T; Takahashi K; Ueno H; Sunaga Y; Yano H; Matsuura Y; Iwanaga T; Takai Y; Seino S. (Department of Molecular Medicine, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan.) NATURE CELL BIOLOGY, (2000 Nov) 2 (11) 805-11. Journal code: DIQ; 100890575. ISSN: 1465-7392. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Although cAMP is well known to regulate exocytosis in many secretory cells, its direct target in the exocytotic machinery is not known. Here we

show that cAMP-**GEFII**, a cAMP sensor, binds to **Rim** (**Rab3**-interacting molecule, **Rab3** being a small **G protein**) and to a new isoform, **Rim2**, both of which are putative regulators of fusion of vesicles to the plasma membrane. We also show that cAMP-**GEFII**, through its interaction with **Rim2** , mediates cAMP-induced, Ca2+-dependent secretion that is not blocked by an inhibitor of **cAMP-dependent** protein kinase (**PKA**). Accordingly, cAMP-**GEFII** is a direct target of cAMP in regulated exocytosis and is responsible for **cAMP-dependent, PKA**-independent exocytosis.

L15 ANSWER 18 OF 62 MEDLINE DUPLICATE 9
2000387713 Document Number: 20344915. PubMed ID: 10884348. Ran alters nuclear pore complex conformation. Goldberg M W; Rutherford S A; Hughes M; Cotter L A; Bagley S; Kiseleva E; Allen T D; Clarke P R. (CRC Department of Structural Cell Biology, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester, M20 9BX, UK.. mgoldberg@picr.man.ac.uk) . JOURNAL OF MOLECULAR BIOLOGY, (2000 Jul 14) 300 (3) 519-29. Journal code: J6V; 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Transport across the nuclear membranes occurs through the nuclear pore complex (NPC), and is mediated by soluble transport factors including Ran, a small GTPase that is generally **GDP**-bound during import and **GTP**-bound for export. The dynamic nature of the NPC structure suggests a possible active role for it in driving translocation. Here we show that RanGTP but not RanGDP causes alterations of NPC structure when injected into the cytoplasm of Xenopus oocytes, including compaction of the NPC and extension of the cytoplasmic filaments. RanGTP caused accumulation of nucleoplasmin-gold along the length of extended cytoplasmic filaments, whereas RanGDP caused accumulation around the cytoplasmic **rim** of the NPC. This suggests a possible role for Ran in altering the conformation of the cytoplasmic filaments during transport.

L15 ANSWER 19 OF 62 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 10
2000:787238 Document No. 134:83853 The transport mechanism of metallothionein is different from that of classical NLS-bearing protein. Nagano, Takayuki; Itoh, Norio; Ebisutani, Chikara; Takatani, Tomoka; Miyoshi, Tomoya; Nakanishi, Tsuyoshi; Tanaka, Keiichi (Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, 565-0871, Japan). J. Cell. Physiol., 185(3), 440-446 (English) 2000. CODEN: JCLLAX. ISSN: 0021-9541. Publisher: Wiley-Liss, Inc..

AB A nuclear localization signal (NLS) has been detected in several nuclear proteins. Classical NLS-mediated nuclear pore targeting is performed by using the cytosolic factors, importin .alpha. and importin .beta., whereas nuclear translocation requires the small GTPase, Ran. In the present study, we demonstrated that nuclear localization of metallothionein (MT) differs from that of classical NLS-mediated substrates. In digitonin-permeabilized BALB/c3T3 cells, biotinylated MT was localized in the nucleus in the presence of ATP and erythrocyte cytosol in the same manner as for SV40 large T NLS-conjugated allophycocyanin (APC-NLS). Under ATP-free conditions, nuclear **rim**-binding was obsd. in both transport substrates. **Rim**-binding of labeled MT was competitively inhibited by the addn. of an excess amt. of unlabeled MT. Different elution profiles were obsd. for the localization-promotion activities of MT in the cytosol compared to those of APC-NLS. Furthermore, nuclear localization of MT was detd. to be a wheat germ agglutinin-insensitive, **GTP**.gamma.S-sensitive, and anti-Ran antibody-sensitive process. Green fluorescent protein-metallothionein (GFP-MT) fusion protein was also localized in the nucleus in the stable transformant of CHL-IU cells. These results strongly suggest that the

targeting by MT of the nuclear pore is mediated by cytosolic factor(s) other than importins and that MT requires Ran for its nuclear localization.

L15 ANSWER 20 OF 62 CAPLUS COPYRIGHT 2001 ACS
2000:466664 Document No. 133:160884 Molecular determinants of presynaptic active zones. Garner, Craig C.; Kindler, Stefan; Gundelfinger, Eckart D. (Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL, 35294-0021, USA). Curr. Opin. Neurobiol., 10(3), 321-327 (English) 2000. CODEN: COPUEN. ISSN: 0959-4388. Publisher: Elsevier Science Ltd..
AB A review with 50 refs. The presynaptic cytoskeletal matrix (cytomatrix) assembled at active zones has been implicated in defining neurotransmitter release sites. Munc 13, Rim, Bassoon and Piccolo/Aczonin are recently identified presynaptic cytomatrix proteins. These multi-domain proteins are thought to organize the exocytotic and endocytic machinery precisely at active zones.

L15 ANSWER 21 OF 62 MEDLINE DUPLICATE 11
2000291080 Document Number: 20291080. PubMed ID: 10828453. The **Rab3**-interacting molecule **RIM** is expressed in pancreatic beta-cells and is implicated in insulin exocytosis. Iezzi M; Regazzi R; Wollheim C B. (Division de Biochimie Clinique, Centre Medical Universitaire, Departement de Medecine Interne, Universite de Geneve, Switzerland.) FEBS LETTERS, (2000 May 26) 474 (1) 66-70. Journal code: EUH; 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB The putative **Rab3** effector **RIM** (**Rab3** -interacting molecule) was detected by Northern blotting, RT-PCR and Western blotting in native pancreatic beta-cells as well as in the derived cell lines INS-1E and HIT-T15. **RIM** was localized on the plasma membrane of INS-1E cells and beta-cells. An involvement of **RIM** in insulin exocytosis was indicated by transfection experiments of INS-1E cells with the **Rab3** binding domain of **RIM**. This domain enhanced glucose-stimulated secretion in intact cells and Ca(2+)-stimulated exocytosis in permeabilized cells. Co-expression of Rab3A reversed the effect of **RIM** on exocytosis. These results suggest an implication of **RIM** in the control of insulin secretion.

L15 ANSWER 22 OF 62 MEDLINE DUPLICATE 12
2001092942 Document Number: 20571323. PubMed ID: 11121524. Immuno-electron microscopic localization of the alpha(1) and beta(1)-subunits of soluble guanylyl cyclase in the guinea pig organ of corti. Heinrich U; Maurer J; Koesling D; Mann W; Forstermann U. (Department of Otolaryngology - Head and Neck Surgery, Johannes Gutenberg University Medical School, 55131, Mainz, Germany.) BRAIN RESEARCH, (2000 Dec 1) 885 (1) 6-13. Journal code: B5L. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.
AB Guanylyl cyclases (GC) catalyze the formation of the intracellular signal molecule cyclic GMP from **GTP**. For some years it has been known that the heme-containing soluble guanylyl cyclase (sGC) is stimulated by NO and NO-containing compounds. The sGC enzyme consists of two subunits (alpha(1) and beta(1)). In the present study, the alpha(1) and beta(1)-subunits were identified in the guinea pig cochlea at the electron microscopic level using a post-embedding immuno-labeling procedure. Ultrathin sections of LR White embedded specimens were incubated with various concentrations of two rabbit polyclonal antibodies to the alpha(1)- and beta(1)-subunit, respectively. The immunoreactivity was visualized by a gold-labeled secondary antibody in an energy-filtering transmission electron microscope (EFTEM). Marked immunoreactivity for both antibodies was found in the inner and outer hair cells, with numerous gold

particles at the border of the cuticular plates, associated with the cell nuclei or attached to electron-dense parts of the cytoplasm. In the pillar cells and apical Deiters cells, soluble guanylyl cyclase immunoreactivity was located at the **rim** of the cuticular plates and between the microtubuli bundles. Together with the recently identified nitric oxide synthase isoforms [Eur. Arch. Otorhinolaryngol. 254 (1997) 396; Eur. Arch. Otorhinolaryngol. 255 (1998) 483], the soluble guanylyl cyclase may be involved in signalling processes in the organ of Corti.

L15 ANSWER 23 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
2001:135178 Document No.: PREV200100135178. Functional interaction of the active zone proteins Munc13-1 and rim suggests molecular link between synaptic vesicle docking and priming. Thakur, P. (1); Betz, A.; Junge, H.; Rosenmund, C.; Brose, N.; Rettig, J.. (1) Max-Planck-Inst Biophysical Chem, Goettingen D Germany. Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-782.2. print. Meeting Info.: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000 Society for Neuroscience. ISSN: 0190-5295. Language: English. Summary Language: English.
AB Synaptic transmission in neurons is restricted to designated release sites, the active zones, at which the final steps of synaptic vesicle exocytosis take place. A small fraction of docked vesicles in any given synapse is primed and ready to fuse in response to elevated calcium levels. In glutamatergic synapses, Munc13-1, a mammalian homologue of C. elegans Unc-13, is essential for priming of synaptic vesicles. The N-terminal zinc finger of **RIM**, a 180 kDa protein specifically localized to active zones, binds to Rab3A and other **Rab3** proteins, and is probably involved in synaptic vesicle docking. By detailed biochemical analysis we demonstrate that the N-terminus of Munc13-1 competes with Rab3A for the same binding site in **RIM**. In electrophysiological experiments, overexpression of the **RIM** -binding region of Munc13-1 in hippocampal primary neurons leads to a strong (70%) reduction in synaptic transmission, which originates from a reduction in the number of primed vesicles. In order to demonstrate the specificity of the function of **RIM**-binding region of Munc13-1 we overexpressed the same peptide in Munc13-1 deficient neurons and observed no difference in synaptic transmitter release. Thus, our data suggest that the interaction between **RIM** and Munc13-1 may functionally couple Rab3A/**RIM**-mediated vesicle docking to Munc13-1 mediated vesicle priming in the active zone. (Supported by the Deutsche Forschungsgemeinschaft)

L15 ANSWER 24 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
2001:75903 Document No.: PREV200100075903. RIM regulates transmitter release and is required for mossy fiber LTP in the hippocampus. Castillo, P. E. (1); Schoch, S.; Malenka, R. C.; Sudhof, T. C.. (1) Stanford University School of Medicine, Stanford, CA USA. Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-6.12. print. Meeting Info.: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000 Society for Neuroscience. ISSN: 0190-5295. Language: English. Summary Language: English.
AB **RIM** (**Rab3**-Interacting Molecule) is a synaptic protein that is located in the plasma membrane close to the active zone. It interacts with the synaptic vesicle protein **Rab3** and is phosphorylated by **PKA**. To explore the physiological role of **RIM** in synaptic vesicle exocytosis and plasticity, we have generated and analyzed mice that lack **RIM**. These mice were viable and fertile without obvious functional impairments. Standard electrophysiological techniques were used to assess synaptic transmission in hippocampal slices in vitro. The input-output function at the Schaffer collateral-pyramidal cell synapse (SCh-CA1) indicated that synaptic efficacy was reduced in **RIM** knockout animals. This appears to be

due to an overall decrease in the probability of transmitter release, as estimated by the run-down in NMDA-EPSCs during MK-801 application. In agreement with this finding, paired-pulse facilitation and post-tetanic potentiation at the SCh-CA1 synapse were both enhanced, whereas long-term potentiation (LTP) was unchanged. In marked contrast, at the mossy fiber-CA3 pyramidal cell synapse short-term plasticity was normal while LTP was abolished. We conclude that **RIM** regulates synaptic vesicle exocytosis at the SCh-CA1 synapse and is essential for mossy fiber LTP. Because Rab3A is also required for this presynaptic long-term plasticity, we hypothesize that modification of the **RIM**-Rab3A interaction promotes a persistent enhancement of evoked glutamate release presumably by increasing the number of docked vesicles and/or facilitating their fusion.

L15 ANSWER 25 OF 62 CAPLUS COPYRIGHT 2001 ACS
1999:113838 Document No. 130:179626 Method and test kit for the diagnosis of Alzheimer's disease using the binding of fluorescent probes to PKC or PKA. Janoshazi, Agnes; De Barry, Jean (Centre National de la Recherche Scientifique (C.N.R.S.), Fr.). PCT Int. Appl. WO 9906590 A1 19990211, 25 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (French). CODEN: PIXXD2. APPLICATION: WO 1998-FR1660 19980727. PRIORITY: FR 1997-9823 19970731.
AB The invention concerns a method for diagnosing Alzheimer's disease by incubating blood samples with a fluorescent probe capable of interacting directly or via another fluorescent probe with PKC or PKA, and measuring the fluorescence intensity under different conditions. Blood samples of Alzheimer's patients and control blood samples where divided in three aliquotes; they were incubated with fim-1; preincubated with staurosporine, a competitive inhibitor at the ATP site, and than with fim-1; or incubated with fim-1 and than with TPA. Samples were excited at 485 nm and fluorescence emission spectra measured; based on the emission maxima, pathol. and control samples could be differentiated. The invention also concerns a test kit comprising the necessary tools and reagents for performing the blood anal.

L15 ANSWER 26 OF 62 MEDLINE DUPLICATE 13
2000012899 Document Number: 20012899. PubMed ID: 10545100. Disruption of Rab3-calmodulin interaction, but not other effector interactions, prevents Rab3 inhibition of exocytosis. Coppola T; Perret-Menoud V; Luthi S; Farnsworth C C; Glomset J A; Regazzi R. (Institut de Biologie Cellulaire et de Morphologie, University of Lausanne, Rue de Bugnon 9, 1005 Lausanne, Switzerland.) EMBO JOURNAL, (1999 Nov 1) 18 (21) 5885-91. Journal code: EMB; 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Rab GTPases regulate membrane traffic between the cellular compartments of eukaryotic cells. **Rab3** is associated with secretory vesicles of neuronal and endocrine cells and controls the Ca(2+)-triggered release of neurotransmitters and hormones. To clarify the mode of action of **Rab3** we generated mutants of the GTPase that do not interact efficiently with its putative effectors Rabphilin and **RIM**. Surprisingly, these mutants transfected in PC12 cells were still capable of inhibiting Ca(2+)-evoked secretion. **Rab3** was shown previously to bind to calmodulin in a Ca(2+)-dependent manner. By replacing two arginines conserved between **Rab3** isoforms, we generated a mutant with a reduced affinity for calmodulin. This mutant retained the capacity to interact with the **Rab3** regulatory proteins, Rabphilin, **RIM**, Mss4 and RabGDI, and was correctly targeted to dense-core secretory granules. However, replacement of the two arginines abolished the ability of the **GTP**-bound form of **Rab3** to inhibit exocytosis of catecholamine- and insulin-secreting cells. We propose that a **Rab3**-calmodulin complex generated by elevated Ca(2+) concentrations mediated at least some of the effects of the GTPase and

c limited the number of exocytotic events that occurred in response to secretory stimuli.

L15 ANSWER 27 OF 62 MEDLINE DUPLICATE 14
2000000127 Document Number: 20000127. PubMed ID: 10532327.
Histamine-modified cationic beta-cyclodextrins as chiral selectors for the enantiomeric separation of hydroxy acids and carboxylic acids by capillary electrophoresis. Galaverna G; Corradini R; Dossena A; Marchelli R. (Dipartimento di Chimica Organica e Industriale, Universita di Parma, Italy.) ELECTROPHORESIS, (1999 Sep) 20 (13) 2619-29. Journal code: ELE; 8204476. ISSN: 0173-0835. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The enantiomeric separation of alpha-hydroxy acids and carboxylic acids was successfully performed by using 6-deoxy-6-N-histamino-beta-cyclodextrin (CD-hm), a monosubstituted positively charged beta-cyclodextrin (beta-CD) bearing a histamine moiety linked to the C6 of a glucose unit in the upper CD **rim** via the amino group. Good results were obtained at a low selector concentration (1 mM). The number of positive charges on the upper **rim** may be modulated as a function of pH, because of the different **pKa** of the amino and the imidazolyl groups, and was found to affect both the enantioselectivity and resolution factors. With the analogous 6-deoxy-[4-(2-aminoethyl)imidazolyl]-beta-cyclodextrin (CD-mh) bearing the histamine moiety linked to the C6 via the imidazolyl group, very poor results were obtained, showing that the proximity of the positive charge to the cavity plays an important role in the enantiomeric recognition. The complexation mode was studied by electrospray ionization-mass spectrometry (ESI-MS) and two-dimensional nuclear magnetic resonance (2-D NMR) ROESY experiments: the recognition model is consistent with an inclusion complexation of the aromatic ring of the analyte within the CD cavity coupled to electrostatic interactions between the carboxylate and the protonated amino group of the cyclodextrin.

L15 ANSWER 28 OF 62 MEDLINE DUPLICATE 15
1999102062 Document Number: 99102062. PubMed ID: 9882455. Introduction of histidine analogs leads to enhanced proton transfer in carbonic anhydrase V. Earnhardt J N; Wright S K; Qian M; Tu C; Laipis P J; Viola R E; Silverman D N. (Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, Florida, 32610-0267, USA.) ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1999 Jan 15) 361 (2) 264-70. Journal code: 6SK; 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB The rate-limiting step in the catalysis of the hydration of CO2 by carbonic anhydrase involves transfer of protons between zinc-bound water and solution. This proton transfer can be enhanced by proton shuttle residues within the active-site cavity of the enzyme. We have used chemical modulation to provide novel internal proton transfer groups that enhance catalysis by murine carbonic anhydrase V (mCA V). This approach involves the site-directed mutation of a targeted residue to a cysteine which is then subsequently reacted with an imidazole analog containing an appropriately positioned leaving group. Compounds examined include 4-bromoethylimidazole (4-BEI), 2-chloromethylimidazole (2-CMI), 4-chloromethylimidazole (4-CMI), and a triazole analog. Two sites in mCA V, Lys 91 and Tyr 131, located on the **rim** of the active-site cavity have been targeted for the introduction of these imidazole analogs. Modification of the introduced Cys 131 with 4-BEI and 4-CMI resulted in enhancements of up to threefold in catalytic activity. The pH profiles indicate the presence of a new proton shuttle residue of **pKa** near 5.8, consistent with the introduction of a functional proton transfer group into the active site. This is the first example of incorporation by chemical modification of an unnatural amino acid analog of histidine that can act as a proton shuttle in an enzyme.

Searched by: Mary Hale 308-4258 CM-1 12D16

L15 ANSWER 29 OF 62 CAPLUS COPYRIGHT 2001 ACS
1998:447008 Document No. 129:172347 Efficient Catalytic Phosphate Diester Cleavage by the Synergetic Action of Two Cu(II) Centers in a Dinuclear Cis-Diaqua Cu(II) Calix[4]arene Enzyme Model. Molenveld, Peter; Engbersen, Johan F. J.; Kooijman, Huub; Spek, Anthony L.; Reinhoudt, David N. (Laboratory of Supramolecular Chemistry and Technology, University of Twente, Enschede, 7500 AE, Neth.). J. Am. Chem. Soc., 120(27), 6726-6737 (English) 1998. CODEN: JACSAT. ISSN: 0002-7863. Publisher: American Chemical Society.

AB A calix[4]arene deriv. functionalized with two cis-diaqua Cu(II) centers at the distal positions of the upper **rim** was synthesized and investigated as a model for dinuclear metalloenzymes that catalyze chem. transformations of phosphate esters. The flexible dinuclear calix[4]arene efficiently catalyzes the transesterification of the RNA model 2-hydroxypropyl-p-nitrophenyl phosphate (HPNP) and the hydrolysis of the DNA model ethyl-p-nitrophenyl phosphate (EPNP) with turnover conversion, thereby exhibiting rate enhancement factors of 1.0.times.104 and 2.7.times.104, resp. The mononuclear ref. complex, lacking the macrocyclic backbone, has a much lower activity, showing that the high catalytic activity of the dinuclear calix[4]arene complex is due to synergetic action of the two Cu(II) centers. Satn. kinetics and pH variation studies point to the formation of a Michaelis-Menten complex in which the phosphate group is doubly Lewis acid activated by coordination to the two Cu(II) centers. In this complex, a Cu(II) bound hydroxide ion, which is present already at pH 6.5, can act as a base in the intramol. transesterification of HPNP or as a nucleophile in the hydrolysis of EPNP. The remarkably low **pKa** of the Cu(II) bound water mols. in the hydrophobic calix[4]arene mimics the low **pKa** of metal bound water mols. in hydrophobic enzyme active sites, which makes the enzyme (model) active under slightly acidic to neutral conditions. The high catalytic efficiency of this enzyme model is attributed to a dynamic binding of the substrate and (pre)transition state, possible by rapid low energy conformational changes of the flexible calix[4]arene backbone.

L15 ANSWER 30 OF 62 MEDLINE DUPLICATE 16
1998213693 Document Number: 98213693. PubMed ID: 9547223.
Calcium-dependent regulation of rab3 in short-term plasticity. Doussau F; Clabecq A; Henry J P; Darchen F; Poulain B. (Laboratoire de Neurobiologie Cellulaire, UPR 9009, Centre National de la Recherche Scientifique, F-67084 Strasbourg Cedex, France.) JOURNAL OF NEUROSCIENCE, (1998 May 1) 18 (9) 3147-57. Journal code: JDF; 8102140. ISSN: 0270-6474. Pub. country: United States. Language: English.

AB The **Rab3** proteins are monomeric **GTP**-binding proteins associated with secretory vesicles. In their active **GTP**-bound state, **Rab3** proteins are involved in the regulation of hormone secretion and neurotransmitter release. This action is thought to involve specific effectors, including two Ca2+-binding proteins, Rabphilin and **Rim**. **Rab3** acts late in the exocytotic process, in a cell domain in which the intracellular Ca2+ concentration is susceptible to rapid changes. Therefore, we examined the possible Ca2+-dependency of the regulatory action of **GTP**-bound **Rab3** and wild-type **Rab3** on neuroexocytosis at identified cholinergic synapses in Aplysia californica. The effects of recombinant GTPase-deficient Aplysia-**Rab3** (apRab3-Q80L) or wild-type apRab3 were studied on evoked acetylcholine release. Intraneuronal application of apRab3-Q80L in identified neurons of the buccal ganglion of Aplysia led to inhibition of neurotransmission; wild-type apRab3 was less effective. Intracellular chelation of Ca2+ ions by EGTA greatly potentiated the inhibitory action of apRab3-Q80L. Train and paired-pulse facilitation, two Ca2+-dependent forms of short-term plasticity induced by a rise in intraterminal Ca2+

concentration, were increased after injection of apRab3-Q80L. This result suggests that the inhibition exerted by **GTP**-bound **Rab3** on neuroexocytosis is reduced during transient augmentations of intracellular Ca2+ concentration. Therefore, a Ca2+-dependent modulation of **GTP**-bound **Rab3** function may contribute to short-term plasticity.

L15 ANSWER 31 OF 62 MEDLINE DUPLICATE 17
1999108172 Document Number: 99108172. PubMed ID: 9889100. Separate nuclear import pathways converge on the nucleoporin Nup153 and can be dissected with dominant-negative inhibitors. Shah S; Forbes D J. (Department of Biology 0347, University of California at San Diego, La Jolla, California 92093-0347, USA.) CURRENT BIOLOGY, (1998 Dec 17-31) 8 (25) 1376-86. Journal code: B44; 9107782. ISSN: 0960-9822. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Proteins generally enter or exit the nucleus as cargo of one of a small family of import and export receptors. These receptors bear distant homology to importin beta, a subunit of the receptor for proteins with classical nuclear localisation sequences (NLSs). To understand the mechanism of nuclear transport, the next question involves identifying the nuclear pore proteins that interact with the different transport receptors as they dock at the pore and translocate through it. RESULTS: Two pathways of nuclear import were found to intersect at a single nucleoporin, Nup153, localized on the intranuclear side of the nuclear pore. Nup153 contains separate binding sites for importin alpha/beta, which mediates classical NLS import, and for transportin, which mediates import of different nuclear proteins. Strikingly, a Nup153 fragment containing the importin beta binding site acted as a dominant-negative inhibitor of NLS import, with no effect on transportin-mediated import. Conversely, a Nup153 fragment containing the transportin binding site acted as a strong dominant-negative inhibitor of transportin import, with no effect on classical NLS import. The interaction of transportin with Nup153 could be disrupted by a non-hydrolyzable form of **GTP** or by a GTPase-deficient mutant of Ran, and was not observed if transportin carried cargo. Neither Nup153 fragment affected binding of the export receptor Crm1 at the nuclear **rim**. CONCLUSIONS: Two nuclear import pathways, mediated by importin beta and transportin, converge on a single nucleoporin, Nup153. Dominant-negative fragments of Nup153 can now be used to distinguish different nuclear import pathways and, potentially, to dissect nuclear export.

L15 ANSWER 32 OF 62 MEDLINE DUPLICATE 18
1998086315 Document Number: 98086315. PubMed ID: 9425005. Region-specific phosphorylation of rabphilin in mossy fiber nerve terminals of the hippocampus. Lonart G; Sudhof T C. (Department of Molecular Genetics and Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, Dallas, Texas 75235, USA.) JOURNAL OF NEUROSCIENCE, (1998 Jan 15) 18 (2) 634-40. Journal code: JDF; 8102140. ISSN: 0270-6474. Pub. country: United States. Language: English.

AB In mossy fiber synapses of the CA3 region of the hippocampus, long-term potentiation (LTP) is induced presynaptically by activation of **cAMP-dependent protein kinase A** (**PKA**). Rab3A is a synaptic vesicle protein that regulates vesicle fusion and is essential for mossy fiber LTP. Rab3A probably acts via two effector proteins, rabphilin and **RIM**, of which rabphilin is an in vitro substrate for **PKA**. To test if rabphilin is phosphorylated in nerve terminals and if its **PKA**-dependent phosphorylation correlates with the **PKA**-dependent induction of LTP in mossy fiber terminals, we have studied the phosphorylation of rabphilin in synaptosomes isolated from the CA1 and CA3 regions of the hippocampus. Rabphilin was phosphorylated in both CA1 and CA3 synaptosomes. However, when we treated the CA1 and CA3 synaptosomes

with forskolin (an agent that enhances **PKA** activity) or induced Ca2+ influx into synaptosomes with high K+, rabphilin phosphorylation was increased selectively in mossy fiber CA3 synaptosomes, but not in CA1 synaptosomes. In contrast, the phosphorylation of synapsin, studied as a control for the specificity of the region-specific phosphorylation of rabphilin, was augmented similarly by both treatments in CA1 and CA3 synaptosomes. These results reveal that the phosphorylation states of two synaptic substrates for **PKA** and CaM KII, rabphilin and synapsin, are regulated differentially in a region-specific manner, an unexpected finding because rabphilin and synapsin are similarly present in CA1 and CA3 synaptosomes and are colocalized on the same synaptic vesicles. The region-specific phosphorylation of rabphilin agrees well with the restricted induction of LTP by presynaptic **PKA** activation in mossy fiber, but not CA1, nerve terminals.

L15 ANSWER 33 OF 62 MEDLINE DUPLICATE 19

97248596 Document Number: 97248596. PubMed ID: 9092582. The 220-kDa rim protein of retinal rod outer segments is a member of the ABC transporter superfamily. Illing M; Molday L L; Molday R S. (Department of Biochemistry and Molecular Biology, University of British Columbia, British Columbia, Vancouver V6T 1Z3, Canada.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Apr 11) 272 (15) 10303-10. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Outer segments of mammalian rod photoreceptor cells contain an abundantly expressed membrane protein that migrates with an apparent molecular mass of 220 kDa by SDS-gel electrophoresis. We have purified the bovine protein by immunoaffinity chromatography, determined its primary structure by cDNA cloning and direct peptide sequence analysis, and mapped its distribution in photoreceptors by immunocytochemical and biochemical methods. The full-length cDNA encodes a 2280-amino acid protein (calculated molecular mass of 257 kDa) consisting of two structurally related, tandem arranged halves. Each half consists of a hydrophobic domain containing six putative transmembrane segments followed by an ATP-binding cassette. A data base homology search showed that the rod outer segment 220-kDa protein is 40-50% identical in amino acid sequence to the ABC1 and ABC2 proteins cloned from a mouse macrophage cell line. Photoaffinity labeling with 8-azido-ATP and nucleotide inhibition studies confirmed that both ATP and **GTP** bind to this protein with similar affinities. Concanavalin A labeling and endoglycosidase H digestion indicated that the rod outer segment protein contains at least one carbohydrate chain. Immunocytochemical and biochemical studies have revealed that the 220-kDa glycoprotein is distributed along the **rim** region and incisures of rod outer segment disc membranes. From these studies we conclude that the 220-kDa glycoprotein of bovine rod outer segment disc membranes or **Rim** ABC protein is a new member of the superfamily of ABC transporters and is the mammalian homolog of the frog photoreceptor **rim** protein.

L15 ANSWER 34 OF 62 MEDLINE DUPLICATE 20

97289718 Document Number: 97289718. PubMed ID: 9144189. Karyopherin beta2 mediates nuclear import of a mRNA binding protein. Bonifaci N; Moroianu J; Radu A; Blobel G. (Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 May 13) 94 (10) 5055-60. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have cloned and sequenced cDNA for human karyopherin beta2, also known as transportin. In a solution binding assay, recombinant beta2 bound directly to recombinant nuclear mRNA-binding protein A1. Binding was inhibited by a peptide representing A1's previously characterized M9 nuclear localization sequence (NLS), but not by a peptide representing a

classical NLS. As previously shown for karyopherin beta1, karyopherin beta2 bound to several nucleoporins containing characteristic peptide repeat motifs. In a solution binding assay, both beta1 and beta2 competed with each other for binding to immobilized repeat nucleoporin Nup98. In digitonin-permeabilized cells, beta2 was able to dock A1 at the nuclear **rim** and to import it into the nucleoplasm. At low concentrations of beta2, there was no stimulation of import by the exogenous addition of the GTPase Ran. However, at higher concentrations of beta2 there was marked stimulation of import by Ran. Import was inhibited by the nonhydrolyzable **GTP** analog guanylyl imidodiphosphate by a Ran mutant that is unable to hydrolyze **GTP** and also by wheat germ agglutinin. Consistent with the solution binding results, karyopherin beta2 inhibited karyopherin alpha/beta1-mediated import of a classical NLS containing substrate and, vice versa, beta1 inhibited beta2-mediated import of A1 substrate, suggesting that the two import pathways merge at the level of docking of beta1 and beta2 to repeat nucleoporins.

L15 ANSWER 35 OF 62 MEDLINE DUPLICATE 21

97272244 Document Number: 97272244. PubMed ID: 9114010. Cloning and characterization of human karyopherin beta3. Yaseen N R; Blobel G. (Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10021, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Apr 29) 94 (9) 4451-6. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Nuclear import of classical nuclear localization sequence-bearing proteins is mediated by karyopherin alpha/beta1 heterodimers. A second nuclear import pathway, mediated by karyopherin beta2 (transportin), recently was described for mRNA-binding proteins. Here we report the cloning and characterization of human karyopherin beta3, which may be involved in a third pathway for nuclear import. Karyopherin beta3 was localized mainly to the cytosol and the nucleus, particularly the nuclear **rim**. It bound to several of the repeat-containing nucleoporins (Nup358, Nup214, Nup153, Nup98, and p62) in overlay and solution-binding assays and was competed away by karyopherin beta1. For Nup98, we localized this binding to the peptide repeat-containing region. Karyopherin beta3 contains two putative Ran-binding homology regions and bound to Ran-**GTP** in a solution-binding assay with much higher affinity than to Ran-**GDP** . Furthermore, it interacted with two ribosomal proteins in an overlay assay. We suggest that karyopherin beta3 is a nuclear transport factor that may mediate the import of some ribosomal proteins into the nucleus.

L15 ANSWER 36 OF 62 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

97153158 EMBASE Document No.: 1997153158. Gonadotropin-releasing hormone and pituitary adenylate cyclase- activating polypeptide affect levels of cyclic adenosine 3',5'-monophosphate- dependent protein kinase A (PKA) subunits in the clonal gonadotrope .alpha.T3-1 cells: Evidence for cross-talk between PKA and protein kinase C pathways. Garrel G.; McArdle C.A.; Hemmings B.A.; Counis R.. Dr. R. Counis, URA CNRS 1449, ECMR, Universite Pierre et Marie Curie, 75252 Paris cedex 05, France. Raymond.counis@snv.jussieu.fr. Endocrinology 138/6 (2259-2266) 1997. Refs: 40.
ISSN: 0013-7227. CODEN: ENDOAO. Pub. Country: United States. Language: English. Summary Language: English.

AB We have shown previously that **protein kinase A** (**PKA**) subunit levels are regulated by activation of **PKA** or protein kinase C (PKC) in anterior pituitary cells. GnRH also influenced **PKA** subunit levels, suggesting that hormonal regulation occurs in gonadotrophs, and therefore, we have reexamined this question using the clonal gonadotrope-derived cell line (.alpha.T3-1 cells). Western blot analysis, using specific immunoaffinity purified immunoglobulins, revealed expression of catalytic (Cat) and regulatory

type I (RI) and type II (RII) subunits of **PKA** in these cells. Activation of adenylyl cyclase (AC) with forskolin, or of PKC with tetradecanoyl phorbol acetate (TPA), caused a rapid (detectable at 0.5-1 h) and concentration-dependent loss of all **PKA** subunits. Forskolin (10-100 .mu.M) reduced Cat and RI by 60% and RII by 30%, whereas TPA (0.1-1 .mu.M) reduced Cat and RII by 50% and RI by 40%. Simultaneous activation of **PKA** and PKC caused the expected dose- dependent reductions in Cat, and the effects of forskolin or TPA were nearly additive. RI and RII were reduced similarly by 10 nM TPA, whereas 100 nm TPA tended to prevent the reduction of RI or RII caused by forskolin. GnRH, which activates phosphoinositidase C and not AC in these cells, caused a clear loss of Cat or RII at all concentrations tested and of RI at 0.1 **riM**. Pituitary adenylate cyclase-activating polypeptide 38, which acts via PVR-1 receptors to stimulate both phosphoinositidase C and AC in these cells, also caused a clear dose-dependent decrease in Cat, RI, and RII, although higher concentrations were needed for the latter effects. Together, the data demonstrate that catalytic and regulatory subunits of **PKA** are subject to both hormonal and receptor-independent regulation in .alpha.T3-1 cells, reinforcing the possibility that such effects occur in nonimmortalized gonadotropes. Whereas the effects of **PKA** activators very likely involve proteolytic degradation of the dissociated **PKA** holoenzyme, the effects of TPA and GnRH occur in the absence of cAMP elevation by unknown mechanisms. Whatever the mechanisms involved, the data reveal a mechanism for cross-talk between phosphoinositidase C and AC-mediated hormonal signals, in which PKC activation seems to play a pivotal role.

L15 ANSWER 37 OF 62 MEDLINE DUPLICATE 22

97394473 Document Number: 97394473. PubMed ID: 9252191. **Rim** is a putative **Rab3** effector in regulating synaptic-vesicle fusion. Wang Y; Okamoto M; Schmitz F; Hofmann K; Sudhof T C. (Department of Molecular Genetics, The University of Texas, Southwestern Medical Center at Dallas, 75235, USA.) NATURE, (1997 Aug 7) 388 (6642) 593-8. Journal code: NSC; 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Rab3** is a neuronal **GTP**-binding protein that regulates fusion of synaptic vesicles and is essential for long-term potentiation of hippocampal mossy fibre synapses. More than thirty Rab **GTP** -binding proteins are known to function in diverse membrane transport pathways, although their mechanisms of action are unclear. We have now identified a putative **Rab3**-effector protein called **Rim** . **Rim** is composed of an amino-terminal zinc-finger motif and carboxy-terminal PDZ and C2 domains. It binds only to **GTP** (but not to **GDP**)-complexed **Rab3**, and interacts with no other Rab protein tested. There is enrichment of **Rab3** and **Rim** in neurons, where they have complementary distributions. **Rab3** is found only on synaptic vesicles, whereas **Rim** is localized to presynaptic active zones in conventional synapses, and to presynaptic ribbons in ribbon synapses. Transfection of PC12 cells with the amino-terminal domains of **Rim** greatly enhances regulated exocytosis in a **Rab3**-dependent manner. We propose that **Rim** serves as a **Rab3**-dependent regulator of synaptic-vesicle fusion by forming a **GTP**-dependent complex between synaptic plasma membranes and docked synaptic vesicles.

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L15 ANSWER 38 OF 62 MEDLINE DUPLICATE 23

97173022 Document Number: 97173022. PubMed ID: 9020890. Ras activation in platelets after stimulation of the thrombin receptor, thromboxane A2 receptor or protein kinase C. Shock D D; He K; Wencel-Drake J D; Parise L V. (Department of Pharmacology, University of North Carolina at Chapel Hill 27599-7365, USA.) BIOCHEMICAL JOURNAL, (1997 Jan 15) 321 (Pt 2) 525-30. Journal code: 9YO; 2984726R. ISSN: 0264-6021. Pub. country:

Searched by: Mary Hale 308-4258 CM-1 12D16

ENGLAND: United Kingdom. Language: English.

AB Several reports have indicated that the small **G-protein** Ras is not present immunologically in platelets. However, here we report the identification of Ras in platelets by immunoprecipitation with the Ras-specific monoclonal antibodies Y13-259 or Y13-238, followed by Western blotting. The presence of Ras was not due to contamination of samples with erythrocytes or leucocytes. Immunofluorescence studies indicated that Ras was present in a peripheral **rim** pattern in fixed, permeabilized platelets, suggesting an intracellular, plasma membrane location. Activation of platelets with the thrombin receptor peptide42-50, the prostaglandin H2/thromboxane A2 mimetic U46619 or phorbol 12-myristate 13-acetate induced a rapid increase in **GTP**-bound, activated Ras. In each case, this increase was inhibited by the protein kinase C (PKC) inhibitor bisindolylmaleimide GF 109203X, suggesting that Ras is activated downstream of PKC in platelets. Thus the activation of Ras in platelets by agonists will now allow consideration of multiple potential Ras-dependent signal transduction pathways in platelet activation processes.

L15 ANSWER 39 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS

1997:468666 Document No.: PREV199799767869. Forskolin enhances glutamate release and phosphorylation of synaptic proteins rabphilin-3a and RIM in mossy fiber enriched hippocampal synaptosomes. Lonart, G. (1); Johnson, K. M.; Sudhof, T. C.. (1) Dep. Mol. Gen., UT Southwestern Med. Sch., Dallas, TX 75235 USA. Society for Neuroscience Abstracts, (1997) Vol. 23, No. 1-2, pp. 363. Meeting Info.: 27th Annual Meeting of the Society for Neuroscience, Part 1 New Orleans, Louisiana, USA October 25-30, 1997 ISSN: 0190-5295. Language: English.

L15 ANSWER 40 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS

1997:291120 Document No.: PREV199799590323. Influence of receptor density on the patterns of beta-2-adrenoceptor desensitization. Rousseau, Guy; Guilbault, Nathalie; Silva, Angelo Da; Mouillac, Bernard; Chidiac, Peter; Bouvier, Michel (1). (1) Dep. Biochimie, Groupe Recherche Systeme Nerveux Autonome, Univ. Montreal, P.O. Box 6128, Sta. centre-ville, Montreal, PQ H3C 3J7 Canada. European Journal of Pharmacology, (1997) Vol. 326, No. 1, pp. 75-84. ISSN: 0014-2999. Language: English.

AB Sustained stimulation of the beta-2-adrenoceptor leads to a desensitization of the receptor-mediated adenylyl cyclase stimulation. While desensitization promoted by nanomolar concentrations of isoproterenol involves the phosphorylation of the beta-2-adrenoceptor by **protein kinase A** alone, both **protein kinase A**- and beta-adrenoceptor kinase-mediated phosphorylation leading to the binding of beta-arrestin contribute to the desensitization evoked by micromolar concentrations of agonist. In the present study, we assessed the influence of receptor density on the patterns of desensitization induced by these two different levels of stimulation. Murine L cells were transfected with a cDNA encoding the human beta-2-adrenoceptor and clonal cell lines expressing various levels of beta-2-adrenoceptor were used for the study. In cell lines expressing the highest number of receptor, approx. 150 000 sites/cell (approx. 3000 fmol/mg of membrane proteins), pretreatment with micromolar concentrations of isoproterenol causes a desensitization pattern characterized by a reduction in both the potency and the efficacy of isoproterenol to further stimulate the adenylyl cyclase activity. In contrast, desensitization induced by 10 **rim** isoproterenol resulted only in a decrease in the potency of isoproterenol. This distinct pattern of desensitization is not seen in cells expressing 12000 receptors/cell (approx. 200 fmol/mg of membrane proteins) and, in that case, pretreatment with 10 nM isoproterenol leads to a reduction in both the sensitivity and the maximal response. Similar effects on the beta-adrenoceptor-stimulated adenylyl cyclase were observed in these cells following treatment with dibutyryl cAMP. Receptor density therefore dramatically influences the pattern of

desensitization evoked by low level of stimulation. The results also demonstrate that although different molecular events are involved in the desensitization evoked by different levels of stimulation, its phenotypic expression can be qualitatively identical in cells expressing a relatively small number of receptors. Hence, **protein kinase A**-mediated desensitization cannot be qualitatively distinguished from the beta-adrenoceptor kinase-mediated process in these cells.

L15 ANSWER 41 OF 62 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 24
1996:643053 Document No. 125:308831 Multiple modes for inclusion complexation between phenobarbital and 2-hydroxypropyl-.beta.-cyclodextrin in aqueous solution. Aki, Hatsumi; Haraguchi, Toru; Niiya, Tokihiro; Goto, Yoshinobu; Yamamoto, Magobei (Faculty Pharmaceutical Sciences, Fukuoka University, Fukuoka, 814-80, Japan). Yakugaku Zasshi, 116(10), 803-812 (Japanese) 1996. CODEN: YKKZAJ. ISSN: 0031-6903.

AB Two inclusion modes were realized in the complexation between phenobarbital (PHB) and 2-hydroxypropyl-.beta.-cyclodextrin (HPCyD) in aq. soln. using 13C-NMR spectroscopic and isothermal titrn. microcalorimetric studies. The geometries of the complexes were estd. by mol. dynamics calcns. Results of 13C-NMR spectrometry showed that the chem. shifts of barbituric acid and Ph rings were significantly shifted upfield due to penetrating both rings into HPCyD cavity. And the affinity consts. of the carbons of the Ph ring were higher than those of the barbituric acid ring. The signals of Et side-chain contrarily moved downfield by the repulsions with the carbons situated around the **rim** of HPCyD. The calorimetric data indicates that 2 different types of PHB-HPCyD complexes at 1:1 stoichiometry are formed in unionized PHB, whereas, single type of inclusion at ionized PHB. The large inflections around **pka** 7.4 were obsd. in all thermodn. parameters. Esp., the values of -.DELTA.G2 were lowered at pH >8.0 due to decreases in the stability consts. to the order of 102 M-1. The barbituric acid ring seems to penetrate the cavity at lower pH than pK1. In both cases, the Et side-chain rests outside of the cavity.

L15 ANSWER 42 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
1995:342227 Document No.: PREV199598356527. A Direct Interaction between G-Protein beta-gamma Subunits and the Raf-1 Protein Kinase. Pumiglia, Kevin M.; Levine, Harry; Haske, Taraneh; Habib, Tania; Jove, Richard; Decker, Stuart J. (1). (1) Parke-Davis Pharmaceuticals, 2800 Plymouth Rd., Ann Arbor, MI 47106 USA. Journal of Biological Chemistry, (1995) Vol. 270, No. 24, pp. 14251-14254. ISSN: 0021-9258. Language: English.

AB Raf-1 is a serine/threonine protein kinase positioned downstream of Ras in the mitogen-activated protein kinase cascade. Using a yeast two-hybrid strategy to identify other proteins that interact with and potentially regulate Raf-1, we isolated a clone encoding the carboxylterminal half of the G-beta-2 subunit of heterotrimeric **G-proteins**. In vitro, purified G-beta-gamma subunits specifically bound to a GST fusion protein encoding amino acids 1-330 of Raf-1 (Raf/330). Binding assays with truncation mutants of GST-Raf indicate that the region located between amino acids 136 and 239 is a primary determinant for interaction with G-beta-gamma. In competition experiments, the carboxyl terminus of beta-adrenergic receptor kinase (beta-ARK) blocked the binding of G-beta-gamma to Raf/330; however, the Raf-1-binding proteins, Ras and 14-3-3, had no effect. Scatchard analysis of in vitro binding between Raf/330 and G-beta-gamma revealed an affinity of interaction (K-d = 163 +- 36 **rim**), similar to that seen between G-beta-gamma and BARK (K-d = 87 +- 24 nM). The formation of native heterotrimeric G-alpha-beta-gamma complexes, as measured by pertussis toxin ADP-ribosylation of G-beta-gamma could be disrupted by increasing amounts of Raf/330, with an EC-50 of approximately 200 nM, in close agreement with the estimated binding affinity. In vivo complexes of Raf-1 and G-beta-gamma were isolated from human embryonic kidney 293-T cells transfected with epitope-tagged

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G-beta-2. The identification and characterization of this novel interaction raises several possibilities for signaling cross-talk between growth factor receptors and those receptors coupled to heterotrimeric **G-proteins**.

L15 ANSWER 43 OF 62 MEDLINE DUPLICATE 25
95273354 Document Number: 95273354. PubMed ID: 7753805. Protein export from the nucleus requires the GTPase Ran and GTP hydrolysis. Moroianu J; Blobel G. (Laboratory of Cell Biology, Howard Hughes Medical Institute, Rockefeller University, New York, NY 10021, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 May 9) 92 (10) 4318-22. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Nuclei of digitonin-permeabilized cells that had been preloaded with a model transport substrate in a cytosol-dependent import reaction were subsequently incubated to investigate which conditions would result in export of transport substrate. We found that up to 80% of the imported substrate was exported when recombinant human Ran and **GTP** were present in the export reaction. Ran-mediated export was inhibited by nonhydrolyzable **GTP** analogs and also by wheat germ agglutinin but was unaffected by a nonhydrolyzable ATP analog. Moreover, a recombinant human Ran mutant that was deficient in its GTPase activity inhibited export. These data indicate that export of proteins from the nucleus requires Ran and **GTP** hydrolysis but not ATP hydrolysis. We also found that digitonin-permeabilized cells were depleted of their endogenous nuclear Ran, thus allowing detection of Ran as a limiting factor for export. In contrast, most endogenous karyopherin alpha was retained in nuclei of digitonin-permeabilized cells. Unexpectedly, exogenously added, fluorescently labeled Ran, although it accessed the nuclear interior, was found to dock at the nuclear **rim** in a punctate pattern, suggesting the existence of Ran-binding sites at the nuclear pore complex.

L15 ANSWER 44 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
1995:229088 Document No.: PREV199598243388. NMR constraints on the location of the retinal chromophore in rhodopsin and bathorhodopsin. Han, May; Smith, Steven O. (1). (1) Dep. Mol. Biophysics Biochem., Yale Univ., 266 Whitney Ave., P.O. Box 208114, New Haven, CT 06520-8114 USA. Biochemistry, (1995) Vol. 34, No. 4, pp. 1425-1432. ISSN: 0006-2960. Language: English.

AB Rhodopsin is the photoreceptor in vertebrate rod cells responsible for vision at low light intensities. The photoreactive chromophore in rhodopsin is 11-cis-retinal bound to the protein via a protonated Schiff base with Glu 113 as the counterion. We have used the observed 13C NMR chemical shifts of the conjugated retinal carbons in combination with semiempirical molecular orbital calculations to establish the major charge interactions in the retinal binding site of rhodopsin and its primary photoproduct, bathorhodopsin. In rhodopsin, the NMR data constrain one of the carboxylate oxygens (O-1) of Glu 113 to be apprx 3 ANG from the C-12 position of the retinal with the second oxygen oriented away from the conjugated retinal chain. The O-1 sbd C-12 sbd H angle is constrained by taking into account the 500 am absorption maximum of the protein-bound retinal as well as the chemical shift data. The bathorhodopsin retinal binding site structure is generated from the rhodopsin model by isomerization of the C-11 dbd C-12 bond and incorporation of C sbd C single bond twists from C-8 to C-15. The resulting structure yields a moderate fit to both the chemical shift data and the 543 **rim** absorption maximum of bathorhodopsin. In both the rhodopsin and bathorhodopsin models, we have included a structural water molecule hydrogen bonded with the Schiff base to account for the high C dbd N stretching vibrations previously observed. Finally, the retinal binding site structure derived from the NMR constraints is used to position the 11-cis-retinal chromophore in the interior of a structural model of the

rhodopsin apoprotein recently proposed on the basis of sequence analysis of **G-protein**-coupled receptors.

L15 ANSWER 45 OF 62 MEDLINE DUPLICATE 26
95247812 Document Number: 95247812. PubMed ID: 7730399. Reassembly of Golgi stacks from mitotic Golgi fragments in a cell-free system. Rabouille C; Misteli T; Watson R; Warren G. (Cell Biology Laboratory, Imperial Cancer Research Fund, London, United Kingdom.) JOURNAL OF CELL BIOLOGY, (1995 May) 129 (3) 605-18. Journal code: HMV; 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Rat liver Golgi stacks were incubated with mitotic cytosol for 30 min at 37 degrees C to generate mitotic Golgi fragments comprising vesicles, tubules, and cisternal remnants. These were isolated and further incubated with rat liver cytosol for 60 min. The earliest intermediate observed by electron microscopy was a single, curved cisterna with tubular networks fused to the cisternal **rims**. Elongation of this cisterna was accompanied by stacking and further growth at the cisternal **rims** . Stacks also fused laterally so that the typical end product was a highly curved stack of 2-3 cisternae mostly enclosing an electron-lucent space. Reassembly occurred in the presence of nocodazole or cytochalasin B but not at 4 degrees C or in the absence of energy supplied in the form of ATP and **GTP**. Pretreatment of the mitotic fragments and cytosol with N-ethylmaleimide (NEM) also prevented reassembly. **GTP** gamma S and AlF prevented reassembly when added during fragmentation but not when added to the reassembly mixture. In fact, **GTP** gamma S stimulated reassembly such that all cisternae were stacked at the end of the incubation and comprised 40% of the total membrane. In contrast, microcystin inhibited stacking so that only single cisternae accumulated. Together these results provide a detailed picture of the reassembly process and open up the study of the architecture of the Golgi apparatus to a combined morphological and biochemical analysis.

L15 ANSWER 46 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
1995:77458 Document No.: PREV199598091758. In Vivo Restriction by LlaI Is Encoded by Three Genes, Arranged in an Operon with llaIM, on the Conjugative Lactococcus Plasmid pTR2030. O'Sullivan, Daniel J.; Zagula, Karen; Klaenhammer, Todd R. (1). (1) Dep. Food Sci., Southeast Diary Foods Res. Cent., N.C. State Univ., Raleigh, NC 27695-7624 USA. Journal of Bacteriology, (1995) Vol. 177, No. 1, pp. 134-143. ISSN: 0021-9193. Language: English.

AB The LlaI restriction and modification (**RIM**) system is encoded on pTR2030, a 46.2-kb conjugative plasmid from Lactococcus lactis. The llaI methylase gene, sequenced previously, encodes a functional type IIS methylase and is located apprx 5 kb upstream from the abiA gene, encoding abortive phage resistance. In this study, the sequence of the region between llaIM and abiA was determined and revealed four consecutive open reading frames (ORFs). Northern (RNA) analysis showed that the four ORFs were part of a 7-kb operon with llaIM and the downstream abiA gene on a separate transcriptional unit. The deduced protein sequence of ORF2 revealed a P-loop consensus motif for ATP/**GTP**-binding sites and a three-part consensus motif for **GTP**-binding proteins. Data bank searches with the deduced protein sequences for all four ORFs revealed no homology except for ORF2 with McrB, in three regions that coincided with the **GTP**-binding motifs in both proteins. To phenotypically analyze the llaI operon, a 9.0-kb fragment was cloned into a high-copy-number lactococcal shuttle vector, pTRKH2. The resulting construct, pTRK370, exhibited a significantly higher level of in vivo restriction and modification in L. lactis NCK203 than the low-copy-number parental plasmid, pTR2030. A combination of deletion constructions and frameshift mutations indicated that the first three ORFs were involved in LlaI restriction, and they were therefore designated llaI.1, llaI.2, and llaI.3. Mutating llaI.1 completely abolished restriction, while disrupting

llaI.2 or llaI.3 allowed an inefficient restriction of phage DNA to occur, manifested primarily by a variable plaque phenotype. ORF4 had no discernible effect on in vivo restriction. A frameshift mutation in llaIM proved lethal to L. lactis NCK203, implying that the restriction component was active without the modification subunit. These results suggested that the LlaI R/M system is unlike any other R/M system studied to date and has diverged from the type IIS class of restriction enzymes by acquiring some characteristics reminiscent of type I enzymes.

L15 ANSWER 47 OF 62 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1994-248443 [30] WPIDS
CR 1994-092376 [11]; 1994-176315 [21]
AB US 5334675 A UPAB: 19940914
A phenolic resin compsn. comprises (a) an acid hardenable phenolic resin; (b) an aryl phosphite of formula (I), where R, X is 6-12C aryl; Y is H, 6-12C aryl, and 1-12C alkyl; and (c) 1-10 wt.% based on the phenolic resin of a cpd. from: an organic nitrogen cpd. with a **pKa** of 0-3; a cpd. with an internal epoxide gp.; and mixts. of these.

Also claimed is a method of hardening the resin compsn. at ambient temp. by the addn. of sufficient water to hydrolyse (I).

USE/ADVANTAGE - The resin compsn. is used in resin concrete, chemically resistant floor overlays, castable refractories, prepregs, **RIM** composites, coated and bonded abrasives, and in coatings for metals, fire retardant fibres, and fabrics. The controlled release of acid by the hydrolysis of (I) provides an extended working time with the resin compsn. at ambient temp. with rapid cure at slightly higher temps.. Dwg.0/0

L15 ANSWER 48 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 27
1994:500109 Document No.: PREV199497513109. Regional distribution and immunocytological localization of red pigment concentrating hormone in the crayfish eyestalk. Rodriguez-Sosa, Leonardo; Calderon, Jesus; Becerra, Elizabeth; Arechiga, Hugo (1). (1) Div. Estudios Posgrado Invest., Fac. Med., UNAM, Circuito Interior, Ciudad Univ., Mex., DFCP 04510 USA. General and Comparative Endocrinology, (1994) Vol. 95, No. 3, pp. 443-456. ISSN: 0016-6480. Language: English.
AB A polyclonal antibody was raised against synthetic tyrosinated crustacean red pigment concentrating hormone (RPCH-Tyr) with the sequence Tyr-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH-2 with a tryptophan amide at the carboxyl terminal end. Its specificity was tested in comparison with peptides of similar structure. It appears to recognize the three to five residues near the carboxyl terminal. Native RPCH in the crayfish eyestalk was determined by two methods: (a) immunoenzymatic assay (ELISA) using the aforementioned antibody; and (b) bioassay on segments of isolated crayfish tegumentary epithelium. The unitary content in whole eyestalks was 5.5 +- 1.0 nmol for samples (n = 18) taken at night. The regional distribution of RPCH content in the eyestalk was determined. The greatest proportion (40%) was found in the sinus gland, and the lowest in the retina plus lamina ganglionaris (6%). The medulla interna, medulla externa, and medulla terminalis contained similar proportions (about 16% each). The highest specific content was in the sinus gland (65.0 vs 24.4 pmol/mu-**g protein** for the whole eyestalk). Immunopositive neurons were identified in the various regions of the eyestalk. In 22 preparations, an average of 7 cells were identified in the ventromedial **rim** of the medulla terminalis, sending axons to the sinus gland, after branching in the neuropil of the medulla terminalis. Dorsally, 2 cells were identified in the medulla interna and 4 large cells and 11 small cells were located in the medulla externa in close proximity to the lamina ganglionaris: none of these cells appeared to project to the sinus gland. Profuse immunopositive fibers were found in the lamina ganglionaris projecting distally toward the base of the retina. Immunopositive axons were also found in the optic nerve.

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L15 ANSWER 49 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS
1993:151597 Document No.: PREV199344070397. Rhodopsin and phototransduction. Hargrave, Paul A.; McDowell, Hugh. Dep. Ophthalmology, Sch. Med., Univ. Fla., Gainesville, Fla. 32610 USA. Friedlander, M. [Editor]; Mueckler, M. [Editor]. International Review of Cytology, (1992) Vol. 137B, pp. 49-97. International Review of Cytology; Molecular biology of receptors and transporters: Receptors. Publisher: Academic Press, Inc. 1250 Sixth Ave., San Diego, California 92101, USA. ISSN: 0074-7696. ISBN: 0-12-364538-7. Language: English.

L15 ANSWER 50 OF 62 CAPLUS COPYRIGHT 2001 ACS
1991:535662 Document No. 115:135662 Synthesis and solvent extraction studies of novel calixarene-based uranophiles bearing hydroxamic groups. Nagasaki, Takeshi; Shinkai, Seiji (Fac. Eng., Kyushu Univ., Fukuoka, 812, Japan). J. Chem. Soc., Perkin Trans. 2 (7), 1063-6 (English) 1991. CODEN: JCPKBH. ISSN: 0300-9580.
GI For diagram(s), see printed CA Issue.
AB Calix[n]arene-based uranophiles [I; n = 4 (II), 6 (III)] bearing hydroxamic groups on the lower **rim** have been synthesized and the extractability (Ex%) and the selectivity towards uranyl ion (UO_2^{2+}) estd. in a two-phase (water-chloroform) solvent extn. system. Ex% for II and III increases from pH 2 and satn. is reached at around pH 5 where 100% extractability occurs. Since the **pKa** values for hydroxamic acids are 8-9, the apparent **pKa** shift caused by the UO_2^{2+}-complexation amts. to 6-7 pK units. Extn. of UO_2^{2+} from aq. carbonate soln. established that III in the org. phase can compete efficiently with CO_3^{2-} ions in the aq. phase for UO_2^{2+} whereas in II and a calix[6]arene-based uranophile bearing six carboxy groups, UO_2^{2+} is reextd. to the aq. phase. The difference indicates that III which has the hexacoordination geometry preorganized for the binding of UO_2^{2+}, is superior to II as a uranophile. The selectivity of III is superior to the uranophile bearing six carboxy groups. The Ex% values for III are scarcely affected by the addn. of competing metal cations (except Fe^{3+}). These results shows that III serves as an excellent UO_2^{2+}-selective extn. reagent.

L15 ANSWER 51 OF 62 CAPLUS COPYRIGHT 2001 ACS
1992:214121 Document No. 116:214121 Special calixarenes, synthesis and properties. Boehmer, Volker (Inst. Org. Chem., Joh. Gutenberg Univ., Mainz, D-6500, Germany). New Sep. Chem. Tech. Radioact. Waste Other Specific Appl. [Proc. Tech. Semin.], 133-41. Editor(s): Cecille, L.; Casarci, M.; Pietrelli, L.. Elsevier: London, UK. (English) 1991. CODEN: 57QAAO.
AB A lecture with 21 refs. The synthesis of well defined calix[4]arenes is described, which consist of different phenolic units, including m-substituted phenols and resorcinol, which contain alkylidene instead of methylene bridges, or which are fixed in the cone-conformation by suitable bridges between two opposite p-positions (at the upper **rim**) or two phenolic oxygen functions (at the lower **rim**). Connection of two or three calix[4]arene subunits in definite ways to larger mol. systems (double or triple calixarenes) was also possible. Some properties of special compds. (**pKa**-values, ion transport through bulk liq. membranes, extn. of alkali picrates) are discussed.

L15 ANSWER 52 OF 62 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1989-114407 [15] WPIDS
CR 1995-328674 [43]
AB WO 8902935 A UPAB: 19991122
A pure polypeptide related to a neutralisinq and/or fusion epitope of Respiratory Syncytial (RS) virus fusion protein is claimed.
Also claimed are pure polypeptides (a) having a mol. wt. of 700-2500

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daltons and sequence Glu-Glu-Val-Leu-Ala-Tyr-Val (I) (b) having a mol. wt. of 700-4000 daltons and sequence Met - Ser - Ile -Ile-Lys-(I)-Val-Gln-Leu-Pro-Leu-Tyr-Gly-Val-Ile-Asp-Thr-Pro-Cys-- Trp-Lys (II) (c) having a mol wt. of 700-4000 daltons and sequence Gln-Gln-Ser-Tyr-Ser-Ile (II) (d) unglycosylated polypeptide related to a neutralising epitope of RS virus G protein.

USE - The polypeptides and nucleotide sequences encoding them can be used for the prodn. of immunogens in vaccine formulations including multivalent vaccines active immunisation and for the qeneration of antibodies for use in passive immunisation, as well as reagents for diagnostic assays.

0/9

Dwg.0/9

ABEQ US 5223254 A UPAB: 19931116

Method for protecting a primate from respiratory syncytial **rims** -induced diseases comprises admin. of a vaccine contg. a pure human RSV fusion protein of mol. wt. 140 kD. Protein is over 75% pure by wt. and had a native dimeric form.

USE/ADVANTAGE - Vaccine against RSV-induced diseases. Also as diagnostic reagents.

Dwg.0/9

ABEQ EP 390799 B UPAB: 19980330

A pure polypeptide related to a neutralisinq and/or fusion epitope of Respiratory Syncytial (RS) virus fusion protein is claimed.

Also claimed are pure polypeptides (a) having a mol. wt. of 700-2500 daltons and sequence Glu-Glu-Val-Leu-Ala-Tyr-Val (I) (b) having a mol. wt. of 700-4000 daltons and sequence Met - Ser - Ile -Ile-Lys-(I)-Val-Gln-Leu-Pro-Leu-Tyr-Gly-Val-Ile-Asp-Thr-Pro-Cys-- Trp-Lys (II) (c) having a mol wt. of 700-4000 daltons and sequence Gln-Gln-Ser-Tyr-Ser-Ile (II) (d) unglycosylated polypeptide related to a neutralising epitope of RS virus **G protein**.

USE - The polypeptides and nucleotide sequences encoding them can be used for the prodn. of immunogens in vaccine formulations including multivalent vaccines active immunisation and for the qeneration of antibodies for use in passive immunisation, as well as reagents for diagnostic assays.

Dwg.0/4

L15 ANSWER 53 OF 62 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 28

1989:510848 Document No.: BA88:126991. EFFECT OF PH AND SALT CONCENTRATION ON BIMODAL INCLUSION OF A NITROXIDE BY CYCLODEXTRINS. KOTAKE Y; JANZEN E G. DEP. CHEM. BIOCHEM., UNIV. GUELPH, GUELPH, ONT. N1G 2W1, CAN.. J AM CHEM SOC, (1989) 111 (19), 7319-7323. CODEN: JACSAT. ISSN: 0002-7863. Language: English.

AB The effect of pH and ionic salts on the binding inclusion complexes between cyclodextrin and a nitroxide radical probe has been examined by using ESR spectroscopy. A sharp decrease in the amount of inclusion relative to the free probe was observed at pH .apprx. 12, which corresponds to the dissociative pH of protons in the hydroxyl groups on the **rim** of the cyclodextrin, indicating that the ionized cyclodextrin does not have the ability to include the nitroxide probe selected. The **pKa**'s of the hydroxyl groups of cyclodextrins are calculated to be 11.3 .+-. 0.8, 11.7 .+-. 0.4, and 11.9 .+-. 0.4 for .alpha.-, .beta.-, and .gamma.-cyclodextrin, respectively, on the basis of the pH dependence of the spectra. When an ionic salt was added to the solution of the complex of .alpha.-, .beta.-, and .gamma.-cyclodextrin, the association constant of the inclusion complex increases as a function of the salt concentration. The nature of the salt effect in the cyclodextrin system is discussed on the basis of the change of the enthalpy and entropy of association by the addition of salt. In addition, as the concentration of the salt is increased the inclusion of the tert-butyl group is enhanced more than that of the phenyl group. This

result is discussed on the basis of the difference in the hydrophobicity of the included group.

L15 ANSWER 54 OF 62 CAPLUS COPYRIGHT 2001 ACS
1988:450503 Document No. 109:50503 Nucleotide binding to the rod outer segment rim protein. Shuster, Terrence A.; Nagy, Agnes K.; Farber, Debora B. (Jules Stein Eye Inst., Los Angeles, CA, 90024, USA). Exp. Eye Res., 46(5), 647-55 (English) 1988. CODEN: EXERA6. ISSN: 0014-4835.
AB Rat rod outer segment proteins were probed with light-activated (azido-labeled) radioactive nucleotides and a nucleotide binding site(s) on the 220-kilodalton rim glycoprotein, which has a preference for guanine nucleotides, was found. Binding to this site is stimulated by the divalent cations Zn2+, Mn2+, and Mg2+ but not Ca2+. This site is under investigation and may play a role in stabilizing protein structure.

L15 ANSWER 55 OF 62 MEDLINE DUPLICATE 29
88255142 Document Number: 88255142. PubMed ID: 3133234. 8-Azido-ATP (alpha 32P) binding to rod outer segment proteins. Shuster T A; Nagy A K; Farber D B. (Jules Stein Eye Institute, UCLA School of Medicine 90024-1771.) EXPERIMENTAL EYE RESEARCH, (1988 Apr) 46 (4) 475-84. Journal code: EPL; 0370707. ISSN: 0014-4835. Pub. country: ENGLAND: United Kingdom. Language: English.
AB ATP has important roles in the vertebrate rod outer segment (ROS) physiological response to light. One of them is the quench of light-activated cGMP-phosphodiesterase activity. How ATP quenches PDE is not established; however, leading hypotheses favor the intervention of a 48-kDa ATP-binding protein and/or an ATP-utilizing rhodopsin kinase in this reaction. We have investigated the binding of [alpha 32P]8-azido-ATP to rat ROS proteins in the presence and absence of various divalent cations and competitive nucleotides. An event we have detected which might further clarify the role of ATP in PDE inactivation is a zinc-induced binding of azido-ATP to rhodopsin. Manganese is also effective in inducing this binding, while magnesium and calcium are not. The azido-ATP binding is eliminated by the addition of ATP, but not **GTP**, UTP, cGMP, or cAMP. A nucleotide-binding site on the **rim** protein is also suggested from these studies.

L15 ANSWER 56 OF 62 MEDLINE DUPLICATE 30
87283897 Document Number: 87283897. PubMed ID: 3302273. Electrostatic effects on modification of charged groups in the active site cleft of subtilisin by protein engineering. Russell A J; Thomas P G; Fersht A R. JOURNAL OF MOLECULAR BIOLOGY, (1987 Feb 20) 193 (4) 803-13. Journal code: J6V; 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The dielectric constant in the active site cleft of subtilisin from Bacillus amyloliquefaciens has been probed by mutating charged residues on the **rim** and measuring the effect on the **pKa** value of the active site histidine (His64) by kinetics. Mutation of a negatively charged surface residue, which is 12 to 13 A from His64, to an uncharged one Asp----Ser99) lowers the **pKa** of the histidine by up to 0.4 unit at low ionic strength (0.005 to 0.01 M). This corresponds to an apparent dielectric constant of about 40 to 50 between Asp99 and His64. The mutation is in an external loop that is known to tolerate a serine at position 99 from homologies with subtilisins from other bacilli. The environment between His64 and Asp99 is predominantly protein. Another charged residue that is at a similar distance from His64 (14 to 15 A) and is also in an external loop that is known to tolerate a serine residue is Glu156, at the opposite side of the active site. There is only water in a direct line between His64 and Glu156. Mutation of Glu----Ser156 also lowers the **pKa** of His64 by up to 0.4 unit at low ionic strength. This change again corresponds to an apparent dielectric constant of about 40 to 50. The **pKa** values were determined from the pH dependence

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of kcat/KM for the hydrolysis of peptide substrates, with a precision of typically +/- 0.02 unit. The following suggests that the changes in **pKa** are real and not artefacts of experimental conditions: Hill plots of the data for **pKa** determination have gradients (h) of -1.00(+/- 0.02), showing that there are negligible systematic deviations from theoretical ionization curves involving a monobasic acid: the pH dependence for the hydrolysis of two different substrates (succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanyl p-nitroanilide and benzoyl-L-valyl-L-glycyl-L-arginyl p-nitroanilide) gives identical results so that the **pKa** is independent of substrate; the pH dependence is unaffected by changing the concentration of enzyme, so that aggregation is not affecting the results; the shift in **pKa** is masked by high ionic strength, as expected qualitatively for ionic shielding of electrostatic interactions.

L15 ANSWER 57 OF 62 MEDLINE DUPLICATE 31
85074460 Document Number: 85074460. PubMed ID: 6096009. Sequential intermediates in the pathway of intercompartmental transport in a cell-free system. Balch W E; Glick B S; Rothman J E. CELL, (1984 Dec) 39 (3 Pt 2) 525-36. Journal code: CQ4; 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.
AB Two-stage incubations and the selective inhibitory effects of N-ethylmaleimide have revealed three steps in the transport of the vesicular stomatitis viral glycoprotein (**G protein**) between compartments of the Golgi. These are "priming" of the donor membrane, making **G protein** available for transfer to the acceptor Golgi stack; "transfer" of **G protein** to the acceptor stack to form a prefusion complex in which **G protein** is still separate from the GlcNAc transferase; and "fusion," the steps that result in the delivery of **G protein** to the same cisternal membranes that contain the GlcNAc transferase. Electron microscopy shows that priming of the donor membrane is accompanied by the formation of a uniform population of small (60-80 nm diameter) vesicles that bud from the **rims** of the cisternae of the Golgi stacks. This suggests the working hypothesis that the above steps correspond to stages in the budding and fusion of transport vesicles.

L15 ANSWER 58 OF 62 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1982-04491J [48] WPIDS
AB US 4359540 A UPAB: 19930915
A polyurethane elastomer is made by injecting into a mould cavity via a **RIM** machine (1) an aromatic polyisocyanate (2) a polyol of above 500 equiv. wt., (3) a chain extending agent comprising a low mol. wt. active hydrogen contg. cpd. having functionality of at least 2, and (4) a catalyst system. (4) contains a polymer made from CH2:CR1COYR (I) where R is a tert. amino gp. with a **pKa** in water of 7.5 or greater; R1 is H or Me; and Y is O or NH.

The catalyst results in improved paintability when using high solids paint. The moulded elastomer is useful for vehicle body parts. (I) does not interfere with the polymer properties or migrate from the polymer.

L15 ANSWER 59 OF 62 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 1982-05122J [49] WPIDS
AB GB 2099439 A UPAB: 19930915
A novel catalyst compsn. comprises a tert. amine-contg. polymer pref. of formula (I) or (II) which contains no active H atoms dissolved or suspended in a liquid which is a reactive component for polyurethane formation.

CH2=C(R')COYR10 (I)

CH2=C(R')COYC(R2R3)m (CR4R5)nCR6R7NR8R9(II)

In the formulae R1 is H or CH3; Y is -O- or NH; R10 is a tert.

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amino-contg. gp. of **pka** at least 7.5 in water; R2-7 are each H, CH3 or alkyl; m=0-1, n=0-6; and R8 and R9 are CH3 or alkyl, or together with attached N form a heterocycle.

The catalyst is esp. used in forming a polyurethane elastomer whereby an aromatic polyisocyanate, a polyol of equiv. wt. 500 an at least bifunctional low mol. wt. active H-contg. chain extender and the catalyst are injected into the desired mould cavity via a **RIM** machine.

The catalyst does not affect the polyurethane properties and the prod. does not lose any polyamine. The **RIM** parts may be coated with high solids enamel paint.

ABEQ GB 2099439 B UPAB: 19930915
A catalyst composition for polyurethane formation which comprises a tertiary amino group containing polymer substantially without active hydrogen atoms dissolved or suspended in a liquid which is a reactive component for polyurethane formation.

L15 ANSWER 60 OF 62 MEDLINE DUPLICATE 32
83057155 Document Number: 83057155. PubMed ID: 6815210. Surfaces of rod photoreceptor disk membranes: integral membrane components. Roof D J; Heuser J E. JOURNAL OF CELL BIOLOGY, (1982 Nov) 95 (2 Pt 1) 487-500. Journal code: HMV; 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB The membrane surfaces within the rod outer segment of the toad, Bufo marinus, were exposed by rapid-freezing followed by freeze-fracture and deep-etching. Platinum-carbon replicas of disk membranes prepared in this way demonstrate a distinct sidedness. The membrane surface that faces the lumen of the disk shows a fine granularity; particles of approximately 6 nm are packed at a density of approximately 30,000/micron 2. These dimensions suggest that the particles represent protrusions of the integral membrane protein, rhodopsin, into the intradisk space. In addition, when rhodopsin packing is intentionally perturbed by exhaustive digestion with phospholipase C, a concomitant change is observed in the appearance of the luminal surface granularity. The cytoplasmic surface of the disk rarely displays this rough texture; instead it exhibits a collection of much larger particles (8-12 nm) present at approximately 10% of the concentration of rhodopsin. This is about the size and concentration expected for certain light-regulated enzymes, cGMP phosphodiesterase and **GTP**-binding protein, which are currently thought to localize on or near the cytoplasmic surface of the disk. The molecular identity of the 8-12-nm particles will be identified in the following companion paper. A further differentiation of the cytoplasmic surface can be seen around the very edge, or **rim**, of each disk. This **rim** has relatively few 8-12-nm particles and instead displays short filamentlike structures connecting it to other membranes. These filaments extend between adjacent disks, across disk incisures, and from disk **rims** to the nearby plasma membrane.

L15 ANSWER 61 OF 62 MEDLINE DUPLICATE 33
78183751 Document Number: 78183751. PubMed ID: 654889. Ultrastructure of the Bunina bodies in anterior horn cells of amyotrophic lateral sclerosis. Tomonaga M; Saito M; Yoshimura M; Shimada H; Tohgi H. ACTA NEUROPATHOLOGICA, (1978 May 24) 42 (2) 81-6. Journal code: 1CE; 0412041. ISSN: 0001-6322. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB Light and electron microscopic studies were made on the anterior horn cells in a case o amyotrophic lateral sclerosis. Eosinophilic inclusions of Bunina type were observed almost selectively in the motor neurons of spinal cord, as well as of brain stem, at the light microscopic level. Fine structural study revealed the presence of two types of cytoplasmic inclusions. The first, mainly corresponding to the light microscopic inclusions, were homogeneous, electron-dense, round- or oval-shaped bodies with vesicular or tubular **rims** and ribosome particles, about 2-5

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mu in diameter, which contained filaments or other cytoplasmic componenets in the clear areas within them. The second were lamellar structures (laminated cytoplasmic bodies, Morales) which appeared to be originating from endoplasmic reticulum. There was no distinct transition in these two types of inclusions and the relationship to each other is not clear. The significance of Bunina body is unknown, but some manifestation of a primary disorder, e.**g.**, **protein** metabolism, rather than a secondary degenerative change in the motor neurons in amyotorophic lateral sclerosis.

L15 ANSWER 62 OF 62 CAPLUS COPYRIGHT 2001 ACS
1959:59779 Document No. 53:59779 Original Reference No. 53:10818e-f Removal of residual gluten from starch. Boie, Hans; Muller, Fritz (Deutsche Maizena Werke G. m. b. H.). DE 950780 19561018 (Unavailable). APPLICATION: DE .
AB Starch solns. contg. 1-2% protein are floated with air, preferably at 50.degree., in a system of 5 vats in series with overflow **rims** for the gluten-starch mixt. which is transported to the surface by the air bubbles. Air is admitted at 0.3-0.5 atm. gage through porous SiO2 filter stones at the bottom of the vats. Thus, a cornstarch suspension contg. 391 g. solids/l. and 6.14 **g. protein**/l. (1.57% protein in the dry solid) was aerated to give 144 g. dry substance and 57.31 **g. protein**/l. (39.80% protein in the dry solids) from the first 3 vats, and 302 g. dry substance and 10 **g. protein**/l. (3.31% I in the dry solid) from the last 2 vats. The purified starch suspension contained 415 g. dry substance and 4.86 **g. protein**/l. (1.17% protein in the dry solids).

=> s isoform(3a)rim or rim2 or rim 2 or regulat? factor(l)vesicle fusion
L16 19 FILE MEDLINE
L17 17 FILE BIOSIS
L18 46 FILE CAPLUS
L19 18 FILE EMBASE
L20 6 FILE JICST-EPLUS
L21 1400 FILE WPIDS

TOTAL FOR ALL FILES
L22 1506 ISOFORM(3A) RIM OR RIM2 OR RIM 2 OR REGULAT? FACTOR(L) VESICLE FUSION

=> s l22 and (rab3 or rab 3 or gdp gtp or gefii or camp dependent or protein kinase a or pka)
L23 2 FILE MEDLINE
L24 2 FILE BIOSIS
L25 5 FILE CAPLUS
L26 2 FILE EMBASE
L27 0 FILE JICST-EPLUS
L28 1 FILE WPIDS

TOTAL FOR ALL FILES
L29 12 L22 AND (RAB3 OR RAB 3 OR GDP GTP OR GEFII OR CAMP DEPENDENT OR PROTEIN KINASE A OR PKA)

=> s l29 not l14
L30 0 FILE MEDLINE
L31 0 FILE BIOSIS
L32 0 FILE CAPLUS
L33 0 FILE EMBASE
L34 0 FILE JICST-EPLUS
L35 0 FILE WPIDS

Searched by: Mary Hale 308-4258 CM-1 12D16

TOTAL FOR ALL FILES
L36 0 L29 NOT L14

=> s l22 and (endocrine or neuroendocrine) and diagnos?
L37 0 FILE MEDLINE
L38 0 FILE BIOSIS
L39 1 FILE CAPLUS
L40 0 FILE EMBASE
L41 0 FILE JICST-EPLUS
L42 1 FILE WPIDS

TOTAL FOR ALL FILES
L43 2 L22 AND (ENDOCRINE OR NEUROENDOCRINE) AND DIAGNOS?

=> dup rem l43
PROCESSING COMPLETED FOR L43
L44 1 DUP REM L43 (1 DUPLICATE REMOVED)

=> d cbib abs

L44 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
2001:261133 Document No. 134:291132 Protein and cDNA sequences of mouse protein **Rim2** and **diagnostic** and therapeutic uses thereof. Seino, Susumu; Shibasaki, Tadao; Ozaki, Nobuaki (JCR Pharmaceuticals Co., Ltd., Japan). Eur. Pat. Appl. EP 1090986 A1 20010411, 42 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2000-116148 20000731. PRIORITY: JP 1999-288372 19991008.

AB The present invention provides protein and cDNA sequences of mouse protein **Rim2**, which is a novel **isoform** of **Rim**, i.e., a protein that interacts with a low mol. G protein Rab3 and is proposed to serve as a regulator of Rab3-dependent synaptic vesicle fusion, and which specifically interacts with the GDP/GTP exchange factor (GEFII; a cAMP sensor). The full-length novel mouse protein **Rim2** sequenced by the present inventors, which consists of 1590 amino acid residues, was found to have 61.6 % identity with rat Rim1. A zinc finger, PDZ and two C2 domains were found highly conserved between Rim1 and **Rim2**. The invention also relates a mutant protein has an amino acid sequence with one or more amino acids deleted, substituted, inserted or added relative to the amino acid sequence of wild type mouse protein **Rim2** and which has a property to interact with GDP/GTP exchange factor II. The present invention further relates to elucidation of the mechanisms of intracellular vesicle transport and secretion, and to the novel protein **Rim2** which is useful in **diagnosis** of **endocrine**-related diseases or neuropathy and in development of agents for prevention and treatment thereof, the gene encoding **Rim2** and an antibody addressed to **Rim2** protein.

=> s protein(a)(rim2 or rim 2)
L45 3 FILE MEDLINE
L46 1 FILE BIOSIS
L47 2 FILE CAPLUS
L48 1 FILE EMBASE
L49 0 FILE JICST-EPLUS
L50 1 FILE WPIDS

TOTAL FOR ALL FILES
L51 8 PROTEIN(A)(RIM2 OR RIM 2)

=> s l51 not (l14 or l29 or l43)

Searched by: Mary Hale 308-4258 CM-1 12D16

L52 2 FILE MEDLINE
L53 1 FILE BIOSIS
L54 1 FILE CAPLUS
L55 1 FILE EMBASE
L56 0 FILE JICST-EPLUS
L57 0 FILE WPIDS

TOTAL FOR ALL FILES
L58 5 L51 NOT (L14 OR L29 OR L43)

=> dup rem l58
PROCESSING COMPLETED FOR L58
L59 3 DUP REM L58 (2 DUPLICATES REMOVED)

=> d cbib abs 1-3;s seino, s?/au,in;s shibasaki, t?/au,in;s ozaki, n?/au,in

L59 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
2000463997 Document Number: 20469042. PubMed ID: 11016827. The rice Rim2 transcript accumulates in response to Magnaporthe grisea and its predicted protein product shares similarity with TNP2-like proteins encoded by CACTA transposons. He Z H; Dong H T; Dong J X; Li D B; Ronald P C. (Department of Plant Pathology, University of California, Davis 95616, USA.) MOLECULAR AND GENERAL GENETICS, (2000 Sep) 264 (1-2) 2-10. Journal code: NGP. ISSN: 0026-8925. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB A rice transcript, Rim2, was identified that accumulated in both incompatible and compatible interactions between rice and Magnaporthe grisea. The Rim2 transcript also accumulated in response to treatment with a cell wall elicitor derived from M. grisea. A 3.3-kb RIM2 cDNA clone was isolated and is predicted to encode a protein of 653 amino acids, which shares 32 55% identity with TNP2-like proteins encoded by CACTA transposons of other plants. A 1.05-kb segment of the Rim2 sequence shows 82% nucleotide sequence identity with sequences flanking the A1 and C members of the rice Xa21 disease resistance gene family. The 5'-upstream region of Rim2 was cloned and the transcriptional start sites were identified. The 5' and 3' noncoding termini of Rim2 are AT-rich. A cis-element showing similarity to a sequence that mediates defense-associated transcriptional activation of the tobacco retrotransposon Tnt1, and four motifs that fit the consensus sequence of the elicitor-responsive elements in the promoters of the parsley PR-1 genes were found in the 5'-upstream region. Four imperfect tandem repeats were identified in the 3' noncoding terminus. Southern analysis with genomic DNA from different rice species indicated that Rim2 is present in 3-4 copies per genome. These results suggest that Rim2 may be one component of a large CACTA-like element, whose transcript accumulates in response to attack by pathogens.

L59 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS
1997:420560 Document No.: PREV199799719763. Epitope tagging to study Rim2p of Saccharomyces cerevisiae, a member of the mitochondrial carrier family. Duyckaerts, C. (1); El Moualij, B.; Soetens, O.; Crabeel, M.; Sluse, F. E.. (1) Univ. Liege, Liege Belgium. FASEB Journal, (1997) Vol. 11, No. 9, pp. A1078. Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology San Francisco, California, USA August 24-29, 1997 ISSN: 0892-6638. Language: English.

L59 ANSWER 3 OF 3 MEDLINE
95198680 Document Number: 95198680. PubMed ID: 7891656. Overexpression of a novel member of the mitochondrial carrier family rescues defects in both DNA and RNA metabolism in yeast mitochondria. Van Dyck E; Jank B; Ragnini A; Schweyen R J; Duyckaerts C; Sluse F; Foury F. (Unite de Biochimie

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Physiologique, Universite Catholique de Louvain, Louvain-la-Neuve, Belgium.) MOLECULAR AND GENERAL GENETICS, (1995 Feb 20) 246 (4) 426-36. Journal code: NGP; 0125036. ISSN: 0026-8925. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The PIF1 and MRS2 gene products have previously been shown to be essential for mitochondrial DNA maintenance at elevated temperatures and mitochondrial group II intron splicing, respectively, in the yeast Saccharomyces cerevisiae. A multicopy suppressor capable of rescuing the respiratory deficient phenotype associated with null alleles of either gene has been isolated. This suppressor is a nuclear gene that was called RIM2/MRS12. The RIM2/MRS12 gene encodes a predicted protein of 377 amino acids that is essential for mitochondrial DNA metabolism and proper cell growth. Inactivation of this gene causes the total loss of mitochondrial DNA and, compared to wild-type rhoo controls, a slow-growth phenotype on media containing glucose. Analysis of the RIM2/MRS12 protein sequence suggests that RIM2/MRS12 encodes a novel member of the mitochondrial carrier family. In particular, a typical triplicate structure, where each repeat consists of two putative transmembrane segments separated by a hydrophilic loop, can be deduced from amino acid sequence comparisons and the hydropathy profile of RIM2/MRS12. Antibodies directed against the aminoterminus of RIM2/MRS12 detect this protein in mitochondria. The function of the RIM2/MRS12 protein and the substrates it might transport are discussed.

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L86 1 FILE WPIDS

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L88 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
2001:261133 Document No. 134:291132 Protein and cDNA sequences of mouse protein Rim2 and diagnostic and therapeutic uses thereof. **Seino, Susumu**; Shibasaki, Tadao; Ozaki, Nobuaki (JCR Pharmaceuticals Co., Ltd., Japan). Eur. Pat. Appl. EP 1090986 A1 20010411, 42 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2000-116148 20000731. PRIORITY: JP 1999-288372 19991008.

AB The present invention provides protein and cDNA sequences of mouse protein Rim2, which is a novel isoform of Rim, i.e., a protein that interacts with a low mol. G protein Rab3 and is proposed to serve as a regulator of Rab3-dependent synaptic vesicle fusion, and which specifically interacts with the GDP/GTP exchange factor (GEFII; a cAMP sensor). The full-length novel mouse protein Rim2 sequenced by the present inventors, which consists of 1590 amino acid residues, was found to have 61.6 % identity with rat Rim1. A zinc finger, PDZ and two C2 domains were found highly conserved between Rim1 and Rim2. The invention also relates a mutant protein has an amino acid sequence with one or more amino acids deleted, substituted, inserted or added relative to the amino acid sequence of wild type mouse protein Rim2 and which has a property to interact with GDP/GTP exchange factor II. The present invention further relates to elucidation of the mechanisms of intracellular vesicle transport and secretion, and to the novel protein Rim2 which is useful in diagnosis of endocrine-related diseases or neuropathy and in development of agents for prevention and treatment thereof, the gene encoding Rim2 and an antibody addressed to Rim2 protein.

L88 ANSWER 2 OF 6 MEDLINE DUPLICATE 2
2001335837 Document Number: 21289222. PubMed ID: 11395774. Regulation of Ca2+ channel expression at the cell surface by the small G-protein kir/Gem. Beguin P; Nagashima K; Gonoi T; **Shibasaki T**; Takahashi K; Kashima Y; **Ozaki N**; Geering K; Iwanaga T; **Seino S**. (Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.) NATURE, (2001 Jun 7) 411 (6838) 701-6. Journal code: NSC; 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

AB Voltage-dependent calcium (Ca2+) channels are involved in many specialized cellular functions, and are controlled by intracellular signals such as heterotrimeric G-proteins, protein kinases and calmodulin (CaM). However, the direct role of small G-proteins in the regulation of Ca2+ channels is unclear. We report here that the GTP-bound form of kir/Gem, identified originally as a Ras-related small G-protein that binds CaM, inhibits high-voltage-activated Ca2+ channel activities by interacting directly

with the beta-subunit. The reduced channel activities are due to a decrease in alpha1-subunit expression at the plasma membrane. The binding of Ca2+/CaM to kir/Gem is required for this inhibitory effect by promoting the cytoplasmic localization of kir/Gem. Inhibition of L-type Ca2+ channels by kir/Gem prevents Ca2+-triggered exocytosis in hormone-secreting cells. We propose that the small G-protein kir/Gem, interacting with beta-subunits, regulates Ca2+ channel expression at the cell surface.

L88 ANSWER 3 OF 6 MEDLINE DUPLICATE 3
2001654647 Document Number: 21564207. PubMed ID: 11707077. Characterization of the Gene EPAC2: Structure, Chromosomal Localization, Tissue Expression, and Identification of the Liver-Specific Isoform. Ueno H; **Shibasaki T**; Iwanaga T; Takahashi K; Yokoyama Y; Liu L M; Yokoi N; **Ozaki N**; Matsukura S; Yano H; **Seino S**. (Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, 1-8-1, Inohana, Chuo-ku, Chiba, 260-8670, Japan.) GENOMICS, (2001 Nov) 78 (1/2) 91-8. Journal code: GEN; 8800135. ISSN: 0888-7543. Pub. country: United States. Language: English.

AB The liver-specific protein cAMP-GEFII (also known as Epac2) belongs to a family of cyclic adenosine monophosphate (cAMP) binding proteins having guanine nucleotide exchange factor (GEF) activity (the cAMP-GEF family). Here we clone the gene EPAC2, encoding cAMP-GEFII, from a human liver cDNA library. Human EPAC2 has at least 31 exons and is mapped to human chromosome 2q31. Analyses by primer extension, reverse transcriptase-polymerase chain reaction, and in situ hybridization revealed the presence of three transcription start sites of liver-specific Epac2: two major sites located in exon 10 and a minor site in intron 9. The same translation start site is used in all three transcripts. Liver-specific cAMP-GEFII protein, which lacks the first cAMP-binding domain and the Dishevelled/Egl-10/Pleckstrin domain, was detected at 79 kDa by immunoblot analysis, confirming the presence of the short form of cAMP-GEFII in the liver. Liver-specific cAMP-GEFII also has GEF activity toward Rap1. These results demonstrate the presence of liver-specific cAMP-GEFII. Together with the previous finding that cAMP-GEFII is responsible for cAMP-dependent exocytosis in secretory cells, our study suggests that cAMP-GEFII may have a distinct role in liver.

L88 ANSWER 4 OF 6 MEDLINE DUPLICATE 4
2001132056 Document Number: 20512528. PubMed ID: 11056535. cAMP-GEFII is a direct target of cAMP in regulated exocytosis. **Ozaki N**; **Shibasaki T**; Kashima Y; Miki T; Takahashi K; Ueno H; Sunaga Y; Yano H; Matsuura Y; Iwanaga T; Takai Y; **Seino S**. (Department of Molecular Medicine, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan.) NATURE CELL BIOLOGY, (2000 Nov) 2 (11) 805-11. Journal code: DIQ; 100890575. ISSN: 1465-7392. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Although cAMP is well known to regulate exocytosis in many secretory cells, its direct target in the exocytotic machinery is not known. Here we show that cAMP-GEFII, a cAMP sensor, binds to Rim (Rab3-interacting molecule, Rab3 being a small G protein) and to a new isoform, Rim2, both of which are putative regulators of fusion of vesicles to the plasma membrane. We also show that cAMP-GEFII, through its interaction with Rim2, mediates cAMP-induced, Ca2+-dependent secretion that is not blocked by an inhibitor of cAMP-dependent protein kinase (PKA). Accordingly, cAMP-GEFII is a direct target of cAMP in regulated exocytosis and is responsible for cAMP-dependent, PKA-independent exocytosis.

L88 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
2000:491569 Document No.: PREV200000491690. The role of pancreatic beta cell ATP-sensitive K+ channels in insulin secretion. **Seino, S. (1)**; **Ozaki, N.**; **Shibasaki, T.**; Yano, H.. (1) Department of

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Molecular Medicine, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba, 260-8670 Japan. Imura, Hiroo; Kasuga, Masato; Nakao, Kazuwa. International Congress Series, (1999) No. 1181, pp. 47-52. International Congress Series; Common disease: Genetic and pathogenetic aspects of multifactorial diseases. print. Publisher: Elsevier Science B.V. Sara Burgerhartstraat 25, 1000 AE, Amsterdam, Netherlands. Meeting Info.: Proceedings of the Uehara Memorial Foundation Symposium on Common Disease Tokyo, Japan June 30-July 02, 1999 ISSN: 0531-5131. ISBN: 0-444-50200-9 (cloth). Language: English. Summary Language: English.

L88 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2001 ACS
2000:101168 Document No. 132:274559 The role of pancreatic .beta. cell ATP-sensitive K+ channels in insulin secretion. **Seino, S.**; **Ozaki, N.**; **Shibasaki, T.**; Yano, H. (Department of Molecular Medicine, Chiba University Graduate School of Medicine, Chiba, 260-8670, Japan). Int. Congr. Ser., 1181(Common Disease: Genetic and Pathogenetic Aspects of Multifactorial Diseases), 47-52 (English) 1999. CODEN: EXMDA4. ISSN: 0531-5131. Publisher: Elsevier Science B.V..
AB The metab. of glucose in pancreatic .beta. cells is the crit. step in glucose-induced insulin secretion. ATP-sensitive K+ channels (KATP channels) play important roles in many cellular functions by coupling cell metab. to elec. activity. We have previously shown that the .beta.-cell KATP channel is composed of four receptors (SUR1) for sulfonylureas, widely used in the treatment of diabetes mellitus, and four inward rectifier K+ channels (Kir6.2). We have examd. the physiol. consequences of genetic disruption of KATP channel function both in transgenic mice expressing a dominant-neg. KATP channel in pancreatic .beta. cells and knock-out mice lacking KATP channels. Our results show that the KATP channel in pancreatic .beta.-cells is a crucial mol. in both glucose-induced and sulfonylurea-induced insulin secretion.

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